A STUDY ON MICROBIOLOGICAL PROFILE OF VAGINITIS AND ITS ASSOCIATION WITH URINARY TRACT INFECTION DURING PREGNANCY IN A TERTIARY CARE HOSPITAL

Dissertation submitted for

M.D. MICROBIOLOGY BRANCH – 1V

DEGREE EXAMINATION

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

CHENNAI – 600 032

TAMILNADU

MAY 2019

CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON MICROBIOLOGICAL PROFILE OF VAGINITIS AND ITS ASSOCIATION WITH URINARY TRACT INFECTION DURING PREGNANCY IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **Dr.G.JABEEN FATHIMA**, during the period of March 2017 to February 2018 under the guidance of **Prof.Dr.S.THASNEEM BANU M.D.,** Professor of Microbiology, Institute of Microbiology , Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai - 600003, in partial fulfillment of the requirement of M.D. MICROBIOLOGY Degree Examination of The Tamilnadu Dr.M.G.R. Medical University to be held in May 2019.

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ACKNOWLEDGEMENTS

I wish to express my sincere thanks to **Dr.R.JAYANTHI,M.D.FRCP (Glas),** Dean, Rajiv Gandhi Government General Hospital & Madras Medical College, Chennai-3 for permitting me to use the resources of this institution for my study.

I express my thanks to **Dr.J.EuphrasiaLatha, M.D.DGO,** Director, Institute of Microbiology for her guidance and support.

Sincere thanks toFormer Professor **Dr.MangalaAdisesh M.D.,** Institute of Microbiology for her constant encouragement and support during this work.

I owe my heartfelt gratitude and sincere thanks to my guide **Dr.S.THASNEEM BANU, M.D.,**Professor, Institute of Microbiology for her valuable suggestions,guidance,constant support, motivation and encouragement throughout this study.

My Sincere thanks to **Dr.T.K.ShaathyGunasingh, M.D.,** Director , Institute of Obstetrics and Gynaecology for her constant encouragement and support during my study.

I would like to thank all my Professors **Dr.U.Umadevi M.D., Dr.R.Vanaja M.D., and Dr.C.P.Ramani M.D.,** for their support during this study.

I extend my gratitude to my co-guide **DR.S.VINOTHA, M.D.,**Assistant Professor, Institute of Microbiology for his valuable guidance and constant support in this study.

I extend my gratitude to my former co-guide **Dr.C.S.SripriyaM.D.,** Senior Assistant Professor, Institute of Microbiology for her valuable guidance and support in this study.

I wish to extend my thanks to our Assistant Professors **Dr.Lakshmipriya.NM.D.DCH., Dr.K.G.Venkatesh M.D., Dr.P.Shanmugapriya M.D., Dr.C.Nithya M.D., Dr.J.PadmaKumariM.D., Dr.R.KesavanM.D.DCH., Dr.B.Gomathi Manu M.D.,** for their support.

I wish to extend my thanks to our former Assistant Professors **Dr.Deepa.R M.D., Dr.K.Ushakrishnan M.D., Dr.N.RathnaPriya M.D., Dr.DavidAgatha M.D., Dr.B.Natesan M.D.DLO.,** for their support.

I would like to extend my thanks to all my postgraduate colleagues and technicians for their constant support and help in this study.

I thank my husband **Dr.A.R.AKBARM.D.S.,**for his constant motivation, emotional support and help in completing the dissertation work. I feel indebted to my father **MR.YOUSUFF GHULAM MOHAMED** and mother **MRS.G.HALEEMA** who had been solid pillars of everlasting support and encouragement and for their heartfelt blessings. I thank my inlaws for their support, blessings and encouragement .I thank my lovable sons **A.R.ASLAM** and **A.R.AKRAM** for their love and sacrifice.

I would like to thank the **Institutional Ethics Committee ,**Madras Medical college for approving my study .

Last but not the least, I would like to thank the patients participated in this study for their co-operation and support.

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Introduction

INTRODUCTION

There is an increasing global burden on lower genital tract infections among women of reproductive age group (both pregnant and non pregnant) . Some of the consequences of these infections are pelvic inflammatory disease, abortions, preterm deliveries and also increasing HIV acquisition . Lower genital tract infections can also lead to complications in pregnancy as well as infections in the new born . These infections are mostly asymptomatic but can lead to serious consequences in their reproductive outcome if left untreated.

Vaginitis refers to a non-specific inflammation of the vagina characterized by watery discharge with burning and itching of the vulva[3]. The three common types of vaginitis includes Bacterial vaginosis (BV), Vulvovaginal candidiasis (VVC) and Trichomoniasis.

BV is a condition characterized by vaginal discharge with raised pH in which normal vaginal flora of Lactobacilli is replaced by a mixed flora of aerobic, anaerobic and microaerophilic species[18].In the past few decades awareness has increased on the importance of bacterial vaginosis in pregnancy, and it has been discovered that pregnant women with BV are at risk of adverse pregnancy outcomes including spontaneous abortion, premature birth, preterm labour, preterm premature rupture of the membranes, amniotic fluid infection, postpartum endometritis, and post cesarean wound infections[6,7]. Therefore BV is an entity

receiving extensive attention during pregnancy and it is found in 15 to 23% of pregnant women with up to 50% of patients being asymptomatic[21,22,23].

In normal healthy female mucosal candidiasis, especially vulvovaginal candidiasis(VVC), is one of the most common fungal diseases[8,9]. Approximately about 75% of the women suffers at least one episode of VVC during their lifetime[10,11]. The causative agent in most cases is Candida albicans[8,10,12]. The most common predisposing factors are pregnancy, diabetes mellitus, and antibiotic treatment[8-11]. Some studies have shown that in the past few decades vulvovaginitis has increased due to the antifungal resistance among the various Candida species and change inthe health quality of women[10,13-15]. Therefore vulvovaginal candidiasis is found to be more common during pregnancy, and pregnant women have higher rates of recurrent infections.

Trichomoniasis is an infection caused by a flagellated protozoon, Trichomonas vaginalis. The antenatal women infected with this parasite may be at risk of unfavourable birth outcomes such as premature rupture of membranes, premature labour, and low birth weight [5]. Trichomoniasis is associated with a 30% increase in low birth weight infants and a 30% increase in risk of preterm births[34]. It is also associated with enhanced predisposition to neoplastic transformation in cervical tissues, infertility and a two fold increased risk of transmission of human immunodeficiency virus (HIV)[34-37] .

Urinary tract infections (UTIs) have a global annual incidence of approximately 150 million cases[4]. It is one of the most common medical complications of pregnancy. During pregnancy the increased incidence of UTI is due to the physiological and the morphological changes that take place in the genitourinary tract[24,25]. Many hormonal and mechanical changes occur in the body during pregnancy[26,27].Starting in the 6th week, with peak incidence during 22nd–24th weeks of gestation, 90% of the pregnant women develop ureteric dilatation which increases the risk of vesicoureteric reflux and urinary stasis[28].Glycosuria and aminoaciduria during pregnancy in addition ,provide an excellent culture medium for the bacteria in areas of urinarystasis[27].These alterations along with already short urethra and difficulty with hygiene due to the distended belly in pregnancy increase the frequency of UTIs in them.

UTI in pregnancy may present either as symptomatic infection or as asymptomatic bacteriuria. The prevalence of asymptomatic bacteriuria range from 2% to 10% in the various studies done globally[29]. The prevalence of UTI (including both symptomatic and asymptomatic bacteriuria) in India in pregnant women is reported to range from 3% to 24%[25,30-33]. UTI in pregnant women can increase the chances to develop anaemia, hypertensive diseases of pregnancy, chronic renal failure, prematurity, and low birth weight babies[39-41]. The upper UTIs in particular may lead to significant morbidity for both the mother and the fetus[30,42].

In the association between UTIs and bacterial vaginosis sexual intercourse has an important confounding role[16,17]. UTI in females develop when uropathogens almost always from the fecal flora colonize the vagina, ascend into the bladder and in some cases into the kidney. Raised vaginal pH because of the reduction in number of lactobacilli which produce lactate and hydrogen peroxide predispose to genitourinary infections[38].UTI and vaginitis during pregnancy have risk to both the mother and the fetus. The lives of both can be saved by a single step of early diagnosis and treatment . This study is aimed to determine the microbiological profile of vaginitis and the association between BV and UTI during pregnancy in a tertiary care hospital.

Aims &objectives

AIMS & OBJECTIVES

AIM:

- To identify common causes of infectious vaginitis in pregnancy and to determine the risk of urinary tract infections in pregnant women with bacterial vaginosis.

Objective:

- To study type of infectious vaginitis during pregnancy.
- To isolate UTI pathogens from urine during pregnancy & determine its antimicrobial susceptibility pattern.
- To determine the association between bacterial vaginosis& UTI in pregnancy.

Review of Literature

REVIEW OF LITERATURE

Lower genital tract infections comprises of infections in the vulva, vagina or cervix. Infections which affect the vulva and the vagina are called as vulvo-vaginitis. Vaginitis is treatable under ordinary conditions but if left untreated can spread up to the upper genital organs leading to pelvic inflammatory disease. Even gynecological procedures like menstrual regulation, insertion of IUDs and induced abortions can lead to upper genital tract infections[43]. These pelvic infections can lead to serious consequences like salpingitis and chronic infections of the pelvis and may later lead on to ectopic pregnancy or infertility.

The etiological agents causing vaginitis can be endogenous or exogenous in origin. The common ones include bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis.

Bacterialvaginosis is a conditions where there is replacement of the normal indigenous vaginal flora by abnormal bacteria. It is most often due to overgrowth of the commensal organisms. It is one of the commonest infection among the lower genital tract infections in the reproductive age group[44]. According to CDC, Bacterial vaginosis is not transmitted sexually but conditions like having multiple partners predispose to its occurrence[46]. The symptoms of bacterial vaginosis are homogenous thin vaginal discharge, lower abdominal pain which may be associated with foul smell[49].

The agents causing bacterial vaginosis are Gardneralla vaginalis, Mobiluncus, Prevotella, Peptostreptococci, and Porphyromonas Species[44]. Overgrowth of these agents causes a reduction in the number of normally occurring microbial flora in the vaginal tract which is responsible for the production of lactic acid (lacto bacilli). The agents mentioned above can occur in the range of 100-1000 times than the normal vaginal microbial organisms[45]. This leads to the development of bacterial vaginosis.

RISK FCTORS OF THE BV[46]

CDC has estimated the following risk factor for bacterial vaginosis

1. Having new sex partner or multiple sex partner
2. Douching

Though bacterial vaginosis is not known to be spread by sexual contact but still women in the sexually active age-group have more predisposition for the occurrence of BV.

The first diagnosis of BV was made by Amsel et al., who studied 397 female students and devised criteria to distinguish non-specific vaginitis from the bacterial vaginosis[21]. He identified that asymptomatic vaginitis was prevalent in more than 50 of students with non-specific vaginitis[21].

AMSEL'S DIAGNOSTIC CRITERIA[21]

1. Homogeneous vaginal discharge
2. Amine odour after the addition of KOH (Whiff test)
3. Presence of Clue cells in gram strain
4. Vaginal pH>4.5

at least 3 out of 4 criteria must be met for the diagnosis of bacterial vaginosis.

Nugent et al. devised another scoring system comparing the occurrence of Lactobacilli, Gardnerella vaginalis or Bacteroides species and curved gram variable bacteria. His scoring system ranged from 0-10 depending upon the occurrence of the above organisms[22].

NUGENT'S SCORING

Score	Lactobacillus Morphotype per field	Gardnerella Morphotype per field	Curved Bacteria (Mobiluncus) Per field
0	>30	0	0
1	5-30	<1	<1-4
2	1-4	1-4	>5
3	<1	5-30	-
4	0	>30	-

SCORING	
0-3	Normal/No Bacterial vaginosis
4-6	Intermediate
7-10	Bacterial vaginosis

Clue cells are vaginal epithelial cells whose cell borders obscured by the adherent small cocco bacilli or gram negative rods or gram variable rods. They represent important criteria in Amsel's criteria for bacterial vaginosis. They can be identified on wet mount and Gram stain. Most often clue cells are obscured by mixed flora consisting of Gram-negative bacilli, Gram-variable bacilli and cocco bacilli[21].

Bacterial vaginosis is an infection of the vagina caused by mainly anaerobic bacteria like Prevotella, Porphyromonas , Peptostreptococci, and Gardnerella[44]. It is a polymicrobial condition caused by mixed organisms. The bacterial vaginosisis also called anaerobic vaginosis, haemophilus, and gardnerella vaginosis. Bacterial vaginosis is significantly associated with many pathological conditions like chorioamnionitis[60].amnionitis[49], endometritis[51] and prematurity. Anaerobic vaginosis represents a disturbance in the vaginal microbial ecosystem.

Bacterial vaginosis is the leading cause associated with the vaginal discharge accounting up to 48% of cases[49]. Prevalence of bacterial vaginosis is estimated to be about 15 to 23% in pregnant women with up to 50% of patients being asymptomatic[21,22,23].

Apart from causing bacterial vaginosis, Gardnerella can also occur as a normal vaginal flora. Studies have shown that there was no difference between the occurrence of gardnerella vaginosis or bacterial vaginosis in sexually active

and virginal women and therefore bacterial vaginosis is not to be considered as a sexually transmitted disease. Around 17.5-29.3% of women going for induced abortion had bacterial vaginosis. Thus bacterial vaginosis is known to be a predisposing factor for miscarriages and pre-term deliveries [22].

Tsirmpa et al showed an association of 5% between Bacterial vaginosis and Trichomoniasis[47]. It is plausible that T. vaginalis infection alters the vaginal ecology and facilitates the development of BV, or that women with BV have lost natural protection against genital tract infections leading to the acquisition of STIs like T. vaginalis infection. Mathivanan et al., showed a significant correlation between bacterial vaginosis and other STIs. Bacterial vaginosis was also found to be more prevalent in patients with tubal infertility(31.5%) than in patients with non-tubal infertility[44]. Prevalence of bacterial vaginosis in female STD patients was 16% more than in the normal population. Similarly, bacterial vaginosis was found in 15% of women prisoners. Higher incidence of bacterial vaginosis was seen in rape victims (50%) and sex workers (70%), the correlation of bacterial vaginosis to uro-gential infections was found to be 19.7% in women of reproductive age-group[49]. The score of greater than 6 by the Nugent's criteria estimates the presence of bacterial vaginosis in infertile women to be around 19%[53]. A score of 4-10 is also found in 39% of infertile women[53]. Thus infertility was found to be correlated with prevalence of bacterial vaginosis. Tubal infertility was found to be significantly associated with women having bacterial vaginosis. Thus bacterial vaginosis is an important cause of tubal infertility. It is

also associated with pre-term deliveries and miscarriages. Thus women having bacterial vaginosis have increased rate of abortions[51].

TREATMENT:

Bacterial vaginosis treatment in asymptomatic pregnant women at high risk (i.e those who have previously delivered a premature infant) for preterm delivery with a recommended oral regimen has reduced preterm delivery in three out of four randomized control trials.

Bacterial Vaginosis in pregnant women is treated with metronidazole 500mg orally twice a day for 7 days or metronidazole 250mg orally three times a day for 7 days or clindamycin 300 mg orally twice a day for 7 days. Since metronidazole is contraindicated in 1st trimester of pregnancy because of possible teratogenic effect , amoxicillin although less effective is suggested as an alternative treatment.

TRICHOMONIASIS

Trichomonas vaginalis is a sexually transmitted protozoan parasite. Every year worldwide around 180 million cases of trichomoniasis occurs[49]. It is a common sexually transmitted infection and occurs as a mixed infection often with other organism like Gonococci. The co-infection rate is around 60%. The spectrum of clinical symptoms can vary from asymptomatic to severe disease. This organism is transmitted mainly by sexual intercourse through coitus from the male partner. It comprises of the following symptoms – yellowish green,

malodorous, frothy, thin vaginal discharge and comprises of signs like burning sensation, itchiness, dysuria and dyspareunia. The classical sign is erythematous vulva and punctuate hemorrhages seen in the vaginal wall. This is called strawberry vagina/cervix[54].

MORPHOLOGY

Among the Trichomonads, Trichomonas vaginalis is the commonly occurring species. The size of the protozoan parasite may range from 7-10 mm[55]. Size and shape may be altered by physio-chemical conditions. But the parasite is mostly seen as pear or oval-shaped in axenic cultures but becomes amoeboid when it attaches to vaginal epithelial cells. It comprises of five flagella, four located on its anterior portion. The fifth flagellum is incorporated inside the undulating membrane which gives it a quivering motility[55]. Nucleus of this parasite is located on its anterior portion. Rod-like structure called axo style bisects the parasite longitudinally and terminates as a sharp point at its posterior end[55]. This structure is responsible for anchorage of the parasite to the vaginal epithelial cells.

CLINICAL MANIFESTATION

Clinical patterns of Trichomoniasis may vary from asymptomatic carrier state to symptomatic disease comprising of vaginal discharge. This parasite infects the squamous epithelium of the vaginal tract. The infection prevails for longer period after the first episode[55]. Trichomoniasis is the disease of the reproductive age-group. Its incubation period varies from 5-28 days. The clinical

picture of the disease can be classified as acute, chronic or asymptomatic[56]. Acute infection presents as copious leucorrhoea. The disease presents with yellow or greenish frothy and mucopurulent vaginal discharge[56]. The disease presents with clinical signs and symptoms which are cyclic and can worsen during menstrual period. Important sign in this disease is the fiery red appearing cervix/vagina which is called as strawberry appearance[57]. This classical description is seen only in 2% of patients. Chronic infection presents with symptoms like pruritus, dyspareunia and absence of discharge. Majority of the patients are asymptomatic and may have normal vaginal pH and normal vaginal flora. After infection around 50% of women develop symptoms within 6 months[58]. The other organ involved in the infection is bartholin's gland. Complications of this disease may include adnexitis, endometritis and pyosalpinx. These complications may lead to cervical erosion and therefore cause infertility and low birth weight. There are many studies showing an increased association between HIV and trichomoniasis[59,60].

MICROSCOPIC AND CULTURE TECHNIQUES

Donne et al., was the first to describe the use of microscope for the observation of Trichomonas in vaginal and cervical secretions[61]. This technique has sensitivity ranging from 38% to as high as 82%[62]. Though this technique is cost-effective but has a low sensitivity since this parasite may lose its motility within few minutes. Thus it may be the best diagnostic test when done at bed side.

The gold standard for the diagnosis of trichmoniasis is broth culture of the organism. This method requires as few as 300-500 parasite per ml of inoculum[63].

Cell culture techniques are also useful for the recovery of Trichomonas vaginalis from clinical specimens as demonstrated by Garber et al., he showed that the cell culture was superior to both broth culture and wet mount.

Stains used are Periodic Acid Schiff (PAS)[65],Acridine orange[64],Leishman, and Fontana. Papanicolaou (PAP) stain also shows significant appeal in the diagnosis of trichomoniasis. Pap stain is routinely used in gynaecological screening of cytological abnormalities.

All staining techniques have considerable limitations because the parasite appears pear-shaped and may resemble polymorphonuclear leucocytes. The typical morphology of the parasite are also lost during fixation of the smears[66]. Antigenic markers of Trichomonas can be demonstrated by immune-blot analysis[67]. Other techniques used for the diagnosis are complement fixation, indirect haemagglutination, gel-diffusion, fluorescent antibody and ELISA. All these techniques detect for the presence of anti-trichomonal antibodies.

TREATMENT

Untill 1959, only topical vaginal preparations were available for trichomoniasis. But topical applications were not useful since they did not penetrate the vaginal epithelium. Even after the treatment the parasite was transmitted form the male partner to the females. Recent available preparations

include clotrimazole, providone iodine and nonoxynol-9, which provide palliative cure. Azomycin antibiotic was found to be highly effective in the treatment of trichomoniasis[68]. They produce nitroimidazole which are cytocidal to the parasite after its diffusion into the organism (Mueller et al.,)[69]. These metabolic products breakdown the DNA strands of the organisms[69]. As a result cell death ensures due to the loss of mitotic activity and the parasite dies within one hour. The cell death is depicted within 8 hours in cell culture.

Metronidazole 250mg given orally for 7 day is the standard treatment for trichomoniasis. Both symptomatic and asymptomatic diseases need treatement[70]. Male partner also has to be treated in order to prevent re-infection. Both partner treatment has a success rate of almost 95% (Heine et al.,)[65].

Trichomonas vaginalis is more common among HIV infected patients. The incidence of trichomoniasis and HIV co-infection ranges between 6-27%. Trichomoniasis also predisposes to other STIs. Its incidence is estimated to be around 15% in STI clinics. In India, prevalence varies between 6 and 10%[71].

Trichomonas vaginalis accounts for 20% of infections in women having vaginitis. Prevalence of about 47% was reported by Manson et al[72], but this is not constant as it may vary from place to place and study to study[72-79]. Microbiological diagnosis is necessary in case of trichomoniasis, a wet mount preparation is sufficient for definitive diagnosis of motile Trichomonas. Thus wet mount is useful in diagnosing about 60% infected women who present with

clinical symptoms of lower genital tract infection. Other staining techniques like papanicolaou and acridine orange are often inaccurate in the diagnosis of the trichomoniasis.

Lemos et al., compared three techniques for the diagnosis of Trichomonas vaginalis in HIV positive and negative women (wet mount, microscopy, culture and cytology). The results were 13.9% by culture, 13.5% by cytology and 11.4% by wet mount.

Weise et al., stated that wet mount microscopy is a highly specific diagnostic method in case of trichomoniasis[79]. It has many advantages like low-cost, most convenient and widely used method for the diagnosis of trichomoniasis in a resource constrained laboratory set up. If examined within ten minutes of collection of sample the results of wet mount are comparable with other techniques. Thus wet mount plays a vital role as a sole screening test in gynecological clinics in the hands of a skilled microscopist. Culture along with wet mount microscopy significantly yields more information. This disease has many medical, social and economic implications. Antenatal complications like pre-mature rupture of membranes, pre-mature labor and low birth weight are known to occur with this infection. Other diseases like cervical cancer, infertility and pelvic inflammatory disease are also associated with this infection.

VULVOVAGINAL CANDIDIASIS

Candida is a common fungus that affects the mucosa, skin, nails and other organs of human body. Vulvo vaginal candidiasis occurs in around 75% of women in reproductive age-group. 40-50% of them have a second attack. Even healthy females grow Candida in SDA cultures. The vaginal tract is colonized with the Candida from the adjacent peri-anal region. By attachment to the vaginal epithelial cells it gains access into the vagina. C. albicans adhere more strongly than non C. albicans[80].

There are many factors associated with vulvovaginal candidiasis like uncontrolled Diabetes mellitus ,pregnancy and oral contraceptive pills. Candida vaginitis is more prevalent in the reproductive age group and rare in pre-menarchial and post-menopausal women. This shows that the candidiasis is highly depended on the influence of hormones. Other factors that predispose to candida are anti-microbial therapy, use of corticosteroids, insertion of intra-uterine devices and increase in the rate of coitus[83]. Colonization and tissue invasion of candiada is depended upon germination. So the factors which enhance the germination (estrogen therapy, pregnancy) increase the chance of Candida vaginitis.

Vulvovaginal candidiasis causes maximum symptoms during the third trimester pregnancy but recurrences can occur throughout pregnancy. This may be due to the high levels of pregnancy hormone that increase the glycogen content in the vagina thereby, providing a rich carbon source for the Candida infection. It

has been found that the adherence of Candida to the vaginal epithelial cells is enhanced by estrogen. Estrogen[87] also enhances yeast to mycelial transformation. Oral contraceptive pills may also cause an increase in Candida vaginitis. Patients with recurrent vulvovaginal candidiasis are to be screened for uncontrolled diabetes mellitus. Thus there is a positive association between vulvovaginal candidiasis and diabetes mellitus but most often this may not be detected by glucose tolerance test. Women taking diet with refined sugar may be predisposed to candida vaginitis.

Sobel et al., stated that vulvovaginal candidiasis is a common cause of morbidity and health related problems in young women[80]. Recent studies have shown that the percentage of non-Candida albicansis on the rise. Carr P L et al, showed that the C. albicansis found as a normal commensal in around 25% of women[82] . More than 75% of women in the reproductive age group will have the incidence of vulvovaginal candidiasis and around half of them will have recurrence. Recurrent candidal vulvovaginitis is defined as four or more symptomatic infections in one year or three episodes of infection not related to the intake of antibiotics occurring within a year[83]. Studies have shown that 10-30% of recurrence is due to non-Candida albicans species. Among them common ones are Candida glabrata, Candida tropicalis, Candida krusei and Candida parapsilosis[84].

Vulvovaginal candidiasis presents as pruritus (50%), vaginal discharge (24%) and dysuria (33%) other symptoms are burning and stinging sensations but these are considered as non-specific symptoms[85,86] .

When a woman presents with candida vulvovaginitis, the following points must be taken in to account:

1. Presence of uncontrolled diabetes mellitus, diet high in refined sugars, use of broad spectrum antibiotics and intake of corticosteriods[87].
2. History of oral and anal sex[88].
3. Factors such a pregnancy, use of oral contraceptives, which indicate state of hyper estrogenism[89].
4. Other dermatological diseases also need to be excluded (allergic dermatitis).
5. Presence of immune-suppressive conditions like HIV and immune suppressive treatments may reduce the local immunity[90].
6. Presence of other infections (trichomoniasis, bacterial vaginosis).
7. Trace elements deficiency like magnesium, zinc, calcium[91].

LAB DIAGNOSIS

A clinical suspicion and proper examination is necessary for the diagnosis. Species like C.albicans, C.tropicalis show the presence of pseudohyphae and mycelia on direct examination in wet mount[92,93]. Culture for Candida is to be performed when there is a discrepancy between clinical suspicion and direct

examination [90, 93]. Now a days culture is mainly performed in commercially available systems. Other methods like latex agglutination and polymerase chain reaction can also be performed. Culture is not always definitive for diagnosis since 25% of asymptomatic women can be colonized by Candida and will grow Candida in culture[94]. So, definitive diagnosis is based on a proper clinical data, physical examination, examination of wet mount, Gram stain and culture. Speciation done by sugar fermentation test,sugar assimilation test and by using media such as CHROMagar candida medium and cornmeal agar.

Growth on SDA:

The colonies appear cream colored ,smooth and pasty.

Gram stain morphology:

Gram positive budding yeast cells with or without pseudohyphae.

Sugar fermentation reactions for Candida species[130]:

Candida species	Glucose	Maltose	Sucrose	Lactose
C.albicans	AG	AG	-	-
C.troplicalis	AG	AG	AG	-
C.kefyer	AG	AG	AG	-
C.guilliermondii	AG	-	AG	-
C.parapsilosis	AG	-	-	-
C.krusei	AG	-	-	-
C.glabrata	AG	-	-	-

A=Acid production , G=Gas production

Sugar assimilation reactions of different Candida species and their growth on TRM[130]:

Candida spp.	Glu	Mal	Suc	Lac	Cel	Gal	Tre	Raf	Mel	Xyl	Ino	Dul	TRM
C.albicans	+	+	+	+	+	+	+	-	-	+	-	-	PP
C.tropicalis	+	+	+	-	+	+	+	-	-	+	-	-	M
C.kefyer	+	-	+	+	+	+	-	+	-	+	-	-	SP
C.parapsilosis	+	+	+	-	-	+	+	-	-	+	-	-	RP
C.guilliermondii	+	+	+	-	+	+	+	+	+	+	-	+	PsP
C.krusei	+	-	-	-	-	-	-	-	-	+	-	-	DP
C.glabrata	+	-	-	-	-	-	+	-	-	+	-	+	PP

Note:Glu=Glucose,Mal=Maltose,Suc=Sucrose,Lac=Lactose,Cel=Cellobiose,Gal=Galactose,Tre=Trehalose,Raf=Raffinose,Mel=Melibiose,Xyl=Xylose, Ino=Inositol , Dul=Dulcitol , +=positive reaction,- = negative reaction.

Tetrazolium Reduction Medium (TRM) :PP=Pale Pink ,OP=Orange Pink, M=Maroon, SP=Salmon Pink, RP=Rose Pink, PsP=Pink and pasty, DP=Pink and dry.

CHROMagar Candida Medium[130]:

It is selective and differential type of chromogenic medium, which is useful for identification of various Candida species . Due to chromogenic substrate in the medium, the colony morphology and color have been well defined when it is used

to isolate the yeasts. The CHROMagar candida shows following colors of colonies after incubation at 30ºc for a period of 48 to 72 hrs:

C.albicans : Light green , C.dubliniensis :Dark green ,C.glabrata :Pink to purple , C.krusei :Pink , C.parapsilosis :Cream to pale pink , C.tropicalis :Blue with pink halo.

Cornmeal agar[130]:

Benham in 1931 described use of cornmeal agar (CMA) for stimulation of chlamydospore formation.

C.albicans: Elongated pseudohyphal cells with large clusters of blastoconidia in junctures between cells, sessile intercalary and many terminal chlamydospores seen.

C.dubliniensis : Clusters of round blastoconidia at the septa, large thick walled terminal chlamydospores.

C.tropicalis: Blastoconidia single or small groups along pseudohyphae. Fir tree appearance.

C.parapsilosis: Short, thin marked curved pseudohyphal cells develop into large giant pseudohyphal cells.

C.krusei:Presence of pseudohyphae with elongated blastoconidia gives match stick appearance.

C.kefyr:Pseudohyphae with elongated blastoconidia which is log in stream appearance.

C.glabrata:Nopseudohyphae,small oval budding yeast cells single terminal budding.

C.lusitaniae: Occasional blastoconidia at septa with short distinctly curved pseudohyphae.

C.guilliermondii: True hyphae absent.Small groups of blastoconidia at septa few short pseudohyphae

TREATMENT

Today the treatment of choice is a single dose of fluconazole 150 mg/ intra-vaginal clotrimazole 100mg daily for 7 days. The side effects of this therapy are gastro-intestinal discomfort, skin rash, headache and tiredness which are tolerated. Azole groups of drugs are known to cause arrhythmias when given along with H1 anti-histamines like astemizole[95].

URINARY TRACT INFECTIONS:

Urinary tract infections (UTIs) in general can be symptomatic or asymptomatic. Symptomatic UTI can be divided into infections restricted to the lower urinary tract (bladder and urethra) , to the upper urinary tract (kidney) or infections with systemic involvement, which is urosepticemia.

DEFINITIONS [50]

Symptomatic Urinary Tract Infection

Cystitis is defined as an inflammation of the urinary bladder. Urethritis is an inflammation of the urethra. Both are most commonly caused by a bacterial infection; in which case, they are also referred to as lower UTIs. Classic symptoms of lower UTIs are dysuria, urinary frequency, and suprapubic pain sometimes in combination with hematuria, but normally without fever. The extent of symptoms varies between different patients and can be very mild to severe. Other diseases can mimic lower urinary tract bacterial infections like vaginitis, interstitial cystitis, and pelvic inflammatory disease.

Asymptomatic Bacteriuria

Asymptomatic bacteriuria refers to bacteriuria inpatients with no clinical UTI symptoms. For women $\geq 10^5$ colony forming units (CFU) per ml in two consecutive clean-catch urine samples is required for the diagnosis of asymptomatic bacteriuria, whereas for men only one clean catch urine sample with $\geq 10^5$ CFU per mL is required—or a single catheterized urine specimen with one single bacterial strain of $\geq 10^2$ CFU per mL in women or men[96].

CLASSIFICATION

Acute cystitis and urethritis can be classified as uncomplicated versus complicated UTI, nosocomial versus community-acquired UTI, and sporadic versus recurrent UTI.

Uncomplicated UTI occurs in persons with normal urinary tract, whereas complicated UTI occurs in individuals with functional or structural changes, implying deteriorated voiding predisposing for bacteriuria.

Nosocomial UTI are infections that occur 48 hours or more after admission to the hospital or as a result of healthcare, whereas community-acquired UTI are UTIs not included in the previous group.

Sporadic UTI include a single UTI treated with antibiotics during 6 months or maximum two UTIs needing antibiotics during 1 year, whereas recurrent UTI comprise atleast two antibiotic treated UTIs during 6 months or three ormore antibiotic treated UTI during 1 year.

Recurrent UTI can be further divided into relapse or reinfection. Relapse infection includes a recurrent infection with the same bacteria as the previous UTI, whereas a reinfection is caused by different bacteria than in the previous infection.

ETIOLOGY

The most common bacterial uropathogen is Escherichia coli , causing more than 80% of UTIs among female ambulatory patients. In men and hospitalized patients, E. coli is still the most commonly isolated bacteria, but with a lower frequency. Other common uropathogenic bacteria include Klebsiella pneumoniae, Proteus mirabilis ,Enterococci, Streptococcus agalactiae, and Staphylococcus

saprophyticus[97,98]. S. saprophyticus is the only urinary pathogen with a seasonal variation, being most common during the late summer and early autumn months[99].

EPIDEMIOLOGY

Prevalence of UTI is different depending on the age and gender of the patient.

During the reproductive period, the gender difference becomes even more pronounced and UTIs are some 50-fold more common in women as compared to men.

Approximately 20% of women between 24 and 64 years old have at least one episode of dysuria each year, most of these being caused by bacterial infections[100].Almost half of all women will experience at least one episode of UTI during their lifetime [101] and about 25% of these have recurrent infections.

PATHOGENESIS[48]

Routes of Infection :

Bacteria can invade and cause a UTI via three major routes: ascending, hematogenous, and lymphatic pathways. Ascending route is the most common course of infection in females, ascent in association with instrumentation (e.g., urinary catheterization, cystoscopy) is the most common cause of hospital-acquired UTIs in both sexes. For UTIs to occur by the ascending pathway, enteric gram-negative bacteria and other microorganisms must be able to colonize the

vaginal cavity or the periurethral area. Once these organisms gain access to the bladder, they multiply and ascend up the ureters to the kidneys. UTIs occur more often in women than men, at least partially because of the short female urethra and its proximity to the anus. Sexual activity can increase chances of bacterial contamination of the female urethra.

UTIs that occur by the hematogenous route spread usually occurs as a result of bacteremia. Any systemic infection can lead to seeding of the kidney, but certain organisms, such as Staphylococcus aureus or Salmonella spp., are particularly invasive. Although most infections involving the kidneys are acquired through the ascending route, yeast (usually Candida albicans), Mycobacterium tuberculosis, Salmonella spp., Leptospira spp., or Staphylococcus aureus in the urine often indicates pyelonephritis acquired via hematogenous spread, or the descending route.

The Host-Parasite Relationship:

Females in particular, are colonized in the vaginal or periurethral area with organisms originating from the gastrointestinal tract, yet they do not develop urinary infections. Whether an organism is able to colonize and then cause a UTI is determined in large part by a complex interplay of host and microbial factors.

Mostly, the host defense mechanisms are able to eliminate the organisms. Urine itself is inhibitory to some of the urethral flora such as anaerobes. In addition, if urine has a low pH, high or low osmolality, high urea concentration, or

high organic acid content, even organisms capable of growth in the urinary tract may be inhibited.

Any interference with the act of normal voiding, such as mechanical obstruction resulting from pregnancy, kidney stones or strictures, will promote the development of UTI.

Valve mechanism at the junction of the ureter and bladder prevents the reflux (backward flow) of urine from the bladder to the upper urinary tract. Therefore, if the function of these valves is inhibited or compromised in any way, such as by obstruction or congenital abnormalities, urine reflux provides a direct route for organisms to reach the kidney.

Hormonal changes associated with pregnancy and their effects on the urinary tract increase the chance for urine reflux into the upper urinary tract.

Activation of the host immune response by uropathogens also plays a key role in fending off infection. For example, bacterial contact with urothelial cells initiates an immune response via a variety of signaling pathways. Bacterial lipopolysaccharide activates host cells to release cytokines such as tumor necrosis factor and interferon-gamma. In addition, bacteria can activate the complement cascade, leading to the production of biologically active components such as opsonins, as well as augment the host's adaptive immune response. Host factors that lead to host susceptibility or resistance to uropathogens have been identified. For example, a glycoprotein synthesized exclusively by epithelial cells in a

specific anatomic location in the kidney, referred to as Tamm-Horsfall protein or uromodulin, serves as an anti-adherence factor by binding to E. coli–expressing type 1 fimbriae .Defensins, a group of small antimicrobial peptides, are produced by a variety of host cells such as macrophages, neutrophils, and cells in the urinary tract and attach to the bacterial cell, eventually causing its death.

Although many microorganisms can cause UTIs, most cases are a result of infection by a few organisms. To illustrate, only a limited number of serogroups of E. coli cause a significant proportion of UTIs. UPEC possesses virulence factors that enhance their ability to colonize and invade the urinary tract. Some of these virulence factors include increased adherence to vaginal and uroepithelial cells by bacterial surface structures (adhesins, in particular, pili), alpha-hemolysin production, and resistance to serum killing activity . Also, genome sequences of some UPEC strains have been determined, indicating that several potential virulence factor genes associated with the acquisition and development of UTIs are encoded on pathogenicity islands (e.g., hemolysins and E. coli P. fimbriae). Uropathogenic E. coli (UPEC) possess pathogenicity islands containing a variety of virulence factors. By definition, pathogenicity islands contain genes that are associated with virulence and are absent from a virulent or less virulent strains of the same species.

The importance of adherence in the pathogenesis of UTIs has also been demonstrated with other species of bacteria. Proteus strains appear to be uniquely suited to cause significant disease in the urinary tract. Data indicate that these

strains are able to facilitate their adherence to the mucosa of kidneys. Also, Proteus is able to hydrolyze urea via urease production. This results in an increase in urine pH that is directly toxic to kidney cells and also stimulates the formation of kidney stones.

Similar findings have been made with Klebsiellaspp. Staphylococcus saprophyticus also adheres better to uroepithelial cells than does S. aureus or S. epidermidis.

Other bacterial characteristics may be important in the pathogenesis of UTIs. Motility may be important for organisms to ascend to the upper urinary tract against the flow of urine and cause pyelonephritis. Some organisms demonstrate greater production of K antigen (capsuleor outer cell wall); this antigen protects bacteria from being phagocytosed.

Finally, despite numerous host defenses and even antibiotic treatments that can effectively sterilize the urine, a significant proportion of patients have recurrent UTIs. Studies show that uropathogens can invade superficial epithelial cells in the bladder and replicate, forming large foci of intracellular E. coli. This invasion of bladder epithelial cells triggers the host immune response, which in turn causes the superficial cells to exfoliate within hours following infection. Although this exfoliation is considered a host defense mechanism by eliminating infected cells, intracellular organisms are able to reemerge from the bladder epithelial cells and invade the underlying, new superficial layer of epithelial cells,

consequently persisting within the urinary tract. Anderson and colleagues reported that intracellular bacteria mature into numerous, large protrusions on the bladder surface they referred to as “pods.” This bacterial organization—in which the intracellular bacteria are embedded in a fibrous, polysaccharide-rich matrix resembling that of a biofilm—may help further explain the persistence of bladder infections despite strong host defenses.

SYMPTOMS

The symptoms of a UTI substantially differ depending on age and type of infection. The adults with urethritis and cystitis typically have frequent and urgent voiding of small volumes of urine and dysuria and nocturia is common. Sensation of lower abdominal discomfort also is a frequent symptom. The urine may be turbid or even bloody in one third of cases[102]. Some infections may progress after 1 or 2 days to develop a clinical picture of upper UTI, including flank or abdominal pain, fever, and vomiting, but acute cystitis seldom progresses to cause septicemia.

SAMPLE COLLECTION

Urine sample must be collected in universal container. Clean the area around urethral opening with soap and clean water then rinse, dry the area with a sterile guazepad ; Hold the labia apart and collect midstream voided urine. For patients with an indwelling urinary catheter the urine sample has to be obtained through the catheter port.

DIAGNOSIS

Examination of urine specimens for bacteriuria and leukocyturia are the primary laboratory investigations in suspected UTI.

Kass suggested that a threshold of $\geq 10^5$ bacteria per mL of urine reliably distinguished contaminated specimens from true bacteriuria in asymptomatic women, and accurately diagnosed women with acute pyelonephritis. Many clinicians subsequently adopted this single criterion to diagnose cystitis, although Kass had not, in fact, studied women with lower tract symptoms[102]. Later, it has become apparent that cystitis with significant bacteriuria and cystitis with lower bacterial counts have a similar pathogenesis and may represent different stages of the same disease[103,104]. Approximately 40% of women who experience symptoms of cystitis have midstream urine cultures containing less than 10^5 bacteria per ml[102,105].

Nowadays, bacterial counts of 10^4 or sometimes even 10^{3}` CFU per ml, depending of the infecting microorganism, are regarded as significant in patients with clinical UTI symptoms. Therefore, the number of bacteria in urine must be interpreted in a complex view together with other laboratory tests and in the whole clinical context. Another laboratory sign of UTI is leukocyturia or pyuria[106]. The finding of pyuria is unfortunately not specific for urethritis and cystitis. Both systemic inflammation and asymptomatic bacteriuria may be accompanied by the presence of leukocytes in urine. Despite its limitations, pyuria

together with other tests serves as one of the indications of infection of the urinary tract[107].

Urine Culture

Conventional microbiologic quantification of bacteriuria is performed by inoculating a predefined urine volume, mostly1 or 10 μl, onto appropriate agar plates, incubating at 37°C overnight, then identifying the bacterial species and estimating the number of bacterial colonies[108]. Antimicrobial susceptibility testing can be performed by subculturing from bacterial colonies.

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a novel technique for rapid identification of bacterial pathogens, possibly also directly from infected urine. Although it gives high accuracy, conventional culture is still needed for bacterial susceptibility testing[108].

Another way of monitoring bacterial growth, especially in the outpatient clinical settings, is the dip slide test. The dip slide consists of a plastic paddle with culture agar on each side that is immersed in urine and then incubated at 37°C overnight. The method allows semi quantitative measurements; however, the accuracy is limited, mainly because of the relatively high inoculums and the small agar surfaces. There are several drawbacks with the method, one being the absence of antibiotic susceptibility testing. Another limitation is the risk of over-interpretation of mixed bacterial cultures, especially gram-negative bacteria and,

conversely, the risk of missing fastidious bacterial growth like Group B Streptococci[109,110].

Microscopic Examination of Urine

Urine can be examined microscopically for the presence of bacteria and leukocytes. Microscopic examination is simple and faster than urine culture. The Gram staining is useful both for analysis of bacterial type, Gram-negatives or-positives, morphology, rod or cocci, as well as for quantitative analysis. The presence of one or more bacteria per oil immersion field in uncentrifuged urine correlates with $\geq 10^5$ bacteria per ml on culture with a sensitivity and specificity over 90%[111]. However, this method does not detect low count infections, nor does it give possibility for species identification or antibiotic resistance.

More than 95% of patients with symptomatic UTIs have significant leukocyturia[112]. However, pyuria is also found in several other diseases not related to UTI. Because examination of centrifuged urine sediment is not reproducible, quantitative analysis of a fresh, uncentrifuged specimen of urine is recommended[106]. The most common method is counting under the microscope using a hemocytometer chamber. A count of 10 or more leukocytes per ml is considered abnormal. Most women with symptomatic lower UTI have more than 60 leukocytes per ml[113]. Leukocyturia must be carefully evaluated together with other symptoms and signs because it lacks specificity for symptomatic UTIs.

Rapid Diagnostic Tests

Several methods are being used for the rapid detection of bacteriuria and leukocyturia. Such methods may be very useful in a clinical setting when a fast diagnosis is essential. Optimally, rapid tests have low cost, high sensitivity, and high specificity. Therefore, they are useful as screening methods for groups at risk, such as pregnant women. Moreover, in the laboratory these tests may also help select which specimens require further microbiologic investigation.

Two biochemical tests have been devised: the nitrite and leukocyte esterase test.

The nitrite test is based on the bacteria's ability to reduce nitrate to nitrite. It is rapid to perform , evaluate and easy to interpret[114,115]. A test strip is immersed in urine and a color change is observed within 2 minutes, if positive. The test has high specificity, but low sensitivity, which implies that positive results indicate prevalence of bacteria, whereas negative results do not rule out bacteriuria because high bacterial concentrations are needed.

Likewise, some common uropathogenic bacteria will not be positive in the test. S.saprophyticus and Enterococci do not reduce nitrate to nitrite and Pseudomonas reduce nitrite further to ammonia and nitrogen—therefore, none of these bacteria will be positive. False-positive results can be obtained after having eaten phenazopyridine, whereas high levels of vitamin C can give false-negative test results.

The Leukocyte esterase test is a simple and rapid test for leukocytes[116]. It has high specificity and sensitivity and gives similar results as microscopy of urine sediment. When granulocytes are available, the test strip rapidly turns purple with intensity corresponding to the leukocyte concentration. It is important to remember that negative results in either or both tests do not exclude bacteriuria and that especially a positive leukocyte esterase test can be due to reasons other than bacterial infection.

TREATMENT

Safe, empiric antibiotic choices in pregnancy for UTI include trimethoprim-sulfamethoxazole 160/800 mg twice daily, nitrofurantoin 100mg twice daily, and cephalexin 250-500mg four times daily[117]. Current guidelines support the use of a 3-day regimen in healthy women[117] or a 7-day regimen to increase the likelihood of definitive cure in women with comorbidities[118]. Successful treatment must also be assured by the acquisition of a negative follow-up urine culture after completion of the antibiotic regimen and periodic screening throughout the remainder of the pregnancy[117].

Materials & Methods

MATERIALS AND METHODS

Study place:

The study was conducted at the Institute of Obstetrics and Gynaecology, Egmore in association with Institute of Microbiology ,Madras Medical College.

Study duration:

One year between March 2017 to February 2018

Study design:

Prospective, Cross sectional study.

Ethical consideration

Approval from the Institutional Ethics committee was obtained before commencement of the study. Informed consent was obtained from all the patients who participated in the study.

Statistical analysis

Statistical analysis were carried out using Statistical packages for social sciences (SPSS). The proportional data of this cross sectional study were analyzed using Pearson's Chi Square analysis test.

Study population:

Inclusion criteria:

Antenatal patients presenting with one or more of following symptoms were included in the study :

- Increased Vaginal discharge with foul smell
- Lower abdominal pain
- Itching or irritation in vagina
- Pain during sexual intercourse
- Dysuria
- Frequent urination with urgency or leaking of urine
- Burning sensation during urination
- Fever with Chills & rigor

Exclusion Criteria:

- Patients on antimicrobial drugs
- Patients with Placenta previa

SAMPLE COLLECTION

A total of 2 high vaginal swabs were taken from each patient using sterile cotton tipped polyester (Himedia) swabs and a 5ml of clean catch midstream urine sample were collected in a sterile universal container.

HIGH VAGINAL SWAB COLLECTION :

After obtaining written informed consent from the patients, a detailed clinical and personal history was taken from the patients.

Specimen collection was done in a well-lighted room. Patients were asked to lie on the examination table in lithotomy position, procedure was explained to the patient and after wearing sterile gloves, speculum was inserted into the vagina. The nature, colour, amount and consistency of the discharge were noted. The swabs were inserted into the vagina and the discharge was collected from the posterior and lateral fornix.

URINE SAMPLE COLLECTION:

Urine sample must be collected in Universal container. Clean the area around urethral opening with soap and clean water then rinse, dry the area with a sterile guazepad ; Hold the labia apart and collect midstream voided urine. For patients with an indwelling urinary catheter the urine sample has to be obtained through the catheter port .

THE SCREENING FOR BACTERIAL VAGINOSIS:

This was done based on Amsel's criteria and Nugent's scoring .

AMSEL'S CRITERIA

It includes homogeneous vaginal discharge, pH of the vagina being > 4.5, the presence of clue cells in wet mount of the vaginal discharge and a positive whiff test.

According to Amsel, if 3 of the 4 criteria are positive, the patient has bacterial vaginosis.

- **Vaginal pH determination**: This was done by dipping pH paper into the secretions pooled in the posterior fornix of vagina and compare the color change with a standardized colorimetric reference chart to estimate the actual vaginal pH .
- **Whiff test**: The secretion from the swab was mixed with a drop of 10% KOH on a clean glass slide. An intense, putrid, fishy odour indicates positive reaction.
- **Wet mount preparation:** Examined for presence of clue cells and motile flagellates of Trichomonas vaginalis.

1. Presence of Clue cells**:** The secretion from the swab was used to prepare a wet mount by mixing the secretions with a drop of normal saline on a clean grease free glass slide ;a cover slip was placed on it. Slide was observed under 10 x & 40 x magnifications within 10 mins.

   The vaginal epithelial cells which were coated with coccobacillary organisms so that their edges which normally have a sharply defined cell border became indistinct or stippled were considered as the clue cells. Clue cells are characteristic feature of BV. If the clue cells constitute 20% or more of the epithelial cells in the high power field it was considered as positive. The clue cells were also seen in Gram stained smear from HVS.

2. Presence of motile flagellates of Trichomonas vaginalis : If wet mount shows presence of motile flagellates then high vaginal swab was cultured on to Diamonds media.

NUGENT'S CRITERIA

The vaginal discharge was smeared on clean glass slides, air dried, heat fixed and stained by Gram's staining.

In this criterion, each Gram-stained smear was evaluated for the following morphotypes under oil immersion objective (l00x) by using the following scheme:

Large gram-positive rods: *Lactobacillus morphotype*.

Small gram-negative rods: *Gardnerellamorphotype*.

Small gram variable rods: *Bacteroidesmorphotype*.

Curved gram variable rods: *Mobilincus morphology*.

The Nugent scoring system for diagnosis of bacterial vaginosis			
Score	Lactobacillus Morphotype per field	Gardnerella Morphotype per field	Curved Bacteria (Mobiluncus) perField
0	>30	0	0
1	5-30	<1	<1-4
2	1-4	1-4	>5
3	<1	5-30	-
4	0	>30	-

Score:

- 0-3 = Normal/No BV
- 4-6 = Intermediate
- 7-10 = Bacterial vaginosis

HVS AND URINE SPECIMEN PROCESSING :

The high vaginal swab was used for aerobic bacterial and fungal culture. The samples were inoculated onto Mac conkey agar, Blood agar and Sabouraud dextrose agar.

Urine samples were inoculated on CLED agar. Semi quantitative culture was done by standard loop method.

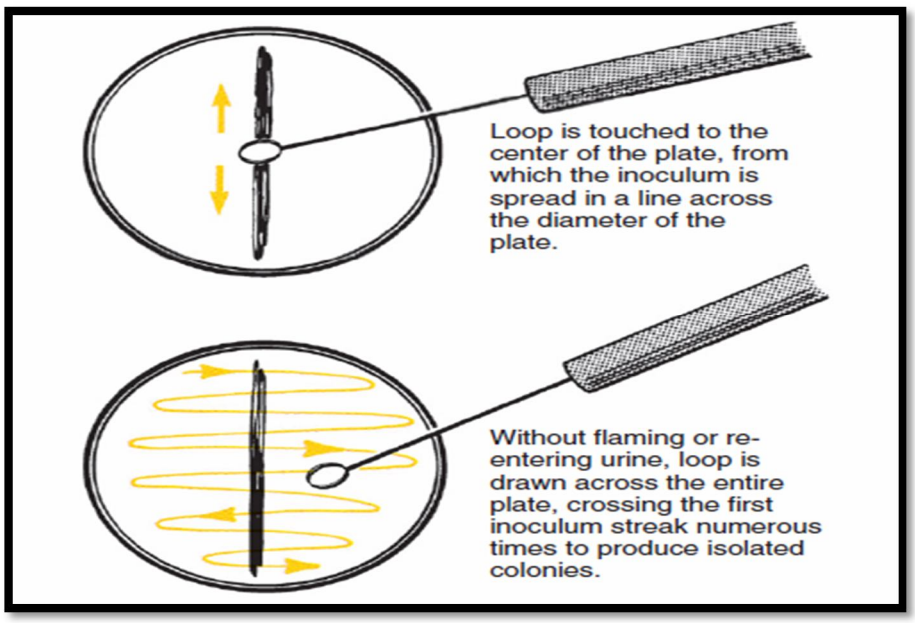
STANDARD LOOP METHOD: The urine specimen was mixed thoroughly before plating. The plates can be inoculated using a calibrated loop to deliver a known volume, 0.01 ml of urine to the surface of CLED agar plate.

Once plated cultures are incubated overnight at 35° C for 24 hours to detect the uropathogens.

No of Colony Forming Units /ml = no. of colonies X100 (4mm loop holding volume of 0.01ml was used)

A count of $\geq 10^5$ colony forming units /ml of urine specimen was considered significant .

The figure given below shows method for streaking with a calibrated loop to produce isolated colonies and countable colony forming units.



IDENTIFICATION OF AEROBIC BACTERIAL ISOLATES:

Isolates from high vaginal swab and urine specimen were identified based on the following identification tests

Identification of isolates of Staphylococcus aureus was based on following characteristics[2]:

1. **Colony morphology:**

   On 5% sheep blood Agar Plate (BAP)- white opaque colonies with a Zone of β-hemolysis is seen.

   On MacConkeyagar (MAC)-Small lactose fermenting opaque colonies .

2. The isolates were subjected to preliminary test like Gram stain, catalase test and coagulase test (both Slide and Tube method).
3. The isolates which were Gram positive cocci in clusters, catalase positive, coagulase test positive (both Slide and Tube method) were subjected to biochemical reactions for further confirmation.
4. The biochemical reactions : Fermentative pattern on Hugh-Leifson's oxidation fermentation media, urea hydrolysis test was positive and mannitol was fermented with gas production.

Identification of isolates of Staphylococcus epidermidis was based on following characteristics[2]:

1. **Colony morphology:**

   On 5% sheep blood Agar Plate (BAP) -non haemolytic white opaque colonies hemolysis is seen.

   On MacConkey agar (MAC)-Small lactose fermenting opaque colonies.
2. The isolates were subjected to preliminary test like Gram stain, catalase test and Coagulase test (both Slide and Tube method).
3. The isolates which were Gram positive cocci in clusters, catalase positive, coagulase test negative (both Slide and Tube method) were subjected to biochemical reactions for further confirmation.
4. The biochemical reactions : Fermentative pattern on Hugh-Leifson's oxidation fermentation media, urea hydrolysis test was positive and mannitol was not fermented.

5. The isolate was sensitive to Novobiocin(30µg) and resistant to Polymyxin B(300IU).

Identification of isolates of Streptococcus agalactiae was based on following characteristics[2]:

1. **Colony morphology:**

   On 5% Blood Agar Plate (BAP)- β haemolytic greyish large (2mm) colonies.

   On MacConkey agar(MAC)-No growth.

2. The isolates were subjected to preliminary test like Gram stain and catalase test.
3. The isolates which were Gram positive cocci in pairs, short chains and catalase negative were subjected to biochemical reactions for further confirmation.
4. The biochemical reactions : Bile aesculin hydrolysis test was negative and Hippurate hydrolysis positive
5. CAMP test positive: when GBS is streaked on BAP perpendicular to S.aureus, an enhanced arrow head shaped hemolysis is produced at their junction, pointing towards GBS streak line.
6. The isolate was resistant to Bacitracin(0.04U) and Cotrimoxazole (1.25/23.75µg).

Identification of isolates of Enterococcus faecalis was based on following characteristics[2]:

1. **Colony morphology**

   On 5% Blood Agar Plate(BAP) - Tiny Translucent non haemolytic colonies.

   On MacConkey agar(MAC)-Small Magenta pink (Lactose Fermenting)colonies
2. The isolates were subjected to preliminary test like Gram stain and catalase test.
3. The isolates which were Gram positive oval cocci in pairs, short chains and catalase negative were subjected to biochemical reactions for further confirmation.
4. The biochemical reactions : Bile aesculin hydrolysis was positive, arginine was dihydrolysed, and arabinose was not fermented.
5. The isolate showed positive heat tolerance test at 60ºC and growth in 6.5% Sodium chloride.

Identification of isolates of family Enterobacteriaceae was based on the following characteristics[2]:

PROPERTIES		**E.coli**	**Klebsiellaspp**	**Proteus spp**
Colony Morphology	**MAC**	Flat lactose fermenting colonies	Large Mucoid lactose fermenting colonies	Non lactose fermenting colonies
	BAP	GWC	GWC	GWC with swarming
Gram stain		GNB	Short plump GNB	Pleomorphic GNB
Catalase		+	+	+

Oxidase	-	-	-
Motility	Motile	Non motile	Motile
Nitrate reduction	Positive	Positive	Positive
Hugh-Leifson'sOf test	Fermentative	Fermentative	Fermentative
Indole	Produced	K.oxytoca-produced K.pneumoniae-notProduced	P.vulgaris-produced P.mirabilis-not Produced
Methyl red test	Positive	Negative	Positive
Voges-proskauer test	Negative	Positive	Negative
Citrate utilisation test	Negative	Positive	Variable
Urea hydrolysis test	Negative	Positive	Positive
Triple sugar iron agar	Acid slant/Acid butt with gas without H_2S	Acid slant/Acid butt with gas without H_2S	Alkaline slant/Acid butt with abundant H_2S
Phenyl pyruvic acid test	Negative	Negative	Positive
Lysine decarboxylation test	Positive	Positive	Negative
Ornithine decarboxylation test	Variable	Negative	P.vulgaris-Negative P.mirabilis-Positive

Identification of isolates ofAcinetobacter baumannii was based on following characteristics[2]:

1. **Colony morphology**

   On MacConkey agar(MAC)-Lactose non- fermenting pale pink colonies

   On 5% sheep blood agar plate(BAP) –small ,circular ,convex smooth colonies with or without hemolysis.

2. The isolates were subjected to preliminary test like Gram stain, catalase test, oxidase test and motility by hanging drop method.
3. The isolates which were Gram negative coccobacilli ,catalase positive, oxidase negative and non motile were subjected to biochemical reactions for further confirmation.
4. The biochemical reactions : Nitrate not reduced , oxidative pattern on Hugh-Leifson's oxidation fermentation media, indole not produced, Citrate utilised, urea not hydrolysed, triple sugar iron agar showed alkaline slant/alkaline butt without gas or H_2S production and 10% OF lactose was fermented.
5. The isolate showed positive growth at 42ºC..

Identification of isolates of Pseudomonas aeruginosa was based on following characteristics[2]:

1. Colony morphology

   On MacConkey agar (MAC)-Large spreading lactose non- fermenting colonies

   On 5%sheep blood agar plate-spreading and flat colonies with serrated edges; with or without hemolysis.
2. The isolates were subjected to preliminary test like Gram stain, catalase test, oxidase test and motility by hanging drop method.
3. The isolates which were Gram negative slender bacilli ,catalase positive, oxidase positive and motile were subjected to biochemical reactions for further confirmation.
4. The biochemical reactions : Nitrate reduced , oxidative pattern on Hugh-Leifson's oxidation fermentation media, indole not produced, Citrate utilised,

urea not hydrolysed, triple sugar iron agar showed alkaline slant/alkaline butt without gas or H_2S production and arginine was dihydrolysed.

5. The isolate showed positive growth at 42ºC..

ANTIBIOTIC SUSCEPTIBILITY TESTING

Disc Diffusion Method

- Antibiotic susceptibility testing of the isolated organisms was done on Mueller Hinton Agar(MHA) plates by Kirby Bauer disc diffusion method as per CLSI document M100,28thedition.The antibiotic discs were obtained from HiMedia Laboratories Private limited, Mumbai.

Zone Diameter Interpretive Standards for *Staphylococcus species*[119]

Antimicrobial Agent (Disc content)	Zone Diameter Interpretive Criteria (nearest whole mm)		
	Sensitive	Intermediate	Resistant
PEN(10 U)	≥ 29	-	≤ 28
COT(1.25/23.75 µg)	≥16	11-15	≤ 10
TET(30 µg)	≥19	15-18	≤ 14
CIP(5µg)	≥21	16-20	≤ 15
ERY(15 µg)	≥23	14-22	≤13
CK(30 µg)	≥18	13-17	≤12
LZ(30 µg)	≥21	-	≤ 20
NIT(300 µg)	≥17	15-16	≤ 14

Nitrofurantoin (NIT)was included for testing and reporting urinary isolates only.

Zone Diameter Interpretive Standards for β haemolytic *Streptococcus species*[119]

Antimicrobial Agent (Disc content)	Zone Diameter Interpretive Criteria (nearest whole mm)		
	Sensitive	Intermediate	Resistant
PEN (10U)	≥ 24	-	-
CTX(30 µg)	≥24	-	-
VAN(30 µg)	≥17	-	-
ERY(15 µg)	≥21	16-20	≤ 15
CK(30 µg)	≥21	18-20	≤ 17

Zone Diameter Interpretive Standards for *Enterococcus faecalis*[119]

Antimicrobial Agent (Disc content)	Zone Diameter Interpretive Criteria (nearest whole mm)		
	Sensitive	Intermediate	Resistant
PEN(10 U)	≥ 15	-	≤ 14
NOR(10 µg)	≥17	13-16	≤ 12
TET(30 µg)	≥19	15-18	≤ 14
CIP(5µg)	≥21	16-20	≤ 15
NIT(300µg)	≥17	15-16	≤ 14
HLG(120 µg)	≥10	7-9	≤ 6

Nitrofurantoin(NIT),Tetracycline(TET),ciprofloxacin(CIP) was included for testing and reporting urinary isolates only. Although Enterococci are often tolerant to β lactams and inherently resistant to aminoglycosides synergistic bactericidal activity results only at high concentration of gentamicin .Hence high level gentamicin (HLG) is used.

Zone Diameter Interpretive Standards for Enterobacteriaceae[119]

Antimicrobial agent (Disc content)	Zone Diameter Interpretive Criteria (nearest whole mm)		
	Sensitive	Intermediate	Resistant
AK(30µg)	≥ 17	15-16	≤ 14
GM(10 µg)	≥15	13-14	≤ 12
COT(1.25/23.75 µg)	≥16	11-15	≤ 10
CIP(5 µg)	≥21	16-20	≤ 15
CTX(30 µg)	≥26	23-25	≤ 22
TET(30 µg)	≥15	12-14	≤ 11
NOR(10 µg)	≥17	13-16	≤ 12
PT (100/10 µg)	≥21	18-20	≤ 17
IMP (10 µg)	≥23	20-22	≤ 19
NIT (300 µg)	≥17	15-16	≤ 14

Nitrofurantoin(NIT) was included for testing and reporting urinary isolates only.

Zone Diameter Interpretive Standards for Gram Negative Non-Fermenter Bacteria[119]

Antimicrobial Agent (Disc content)	Gram Negative Bacilli	Zone Diameter Interpretive Criteria(nearest whole mm)		
		Sensitive	Intermediate	Resistant
GM(10 µg)	P.aeruginosa and A. baumannii	≥15	13-14	≤ 12
COT(1.25/23.75 µg)	A.baumannii	≥16	11-15	≤ 10
CIP(5 µg)	P.aeruginosa and A. baumannii	≥21	16-20	≤ 15
CAZ(30 µg)	P.aeruginosa and A. baumannii	≥18	15-17	≤ 14
TET (30 µg)	A. baumannii	≥15	12-14	≤ 11
NOR(10 µg)	P. aeruginosa	≥17	13-16	≤ 12
PT(100/10 µg)	A.baumannii	≥21	18-20	≤ 17
	P. aeruginosa	≥21	15-20	≤ 14
IMP(10 µg)	P. aeruginosa	≥19	16-18	≤ 15
	A.baumannii	≥22	19-21	≤ 18

Tetracycline (TET) was included for testing and reporting urinary isolates only.

DETECTION OF ANTIMICROBIAL RESISTANCE MECHANISMS:

Detection of Methicillin Resistance in Staphylococcus aureus by using Disc diffusion method[119] :

- Inoculum preparation : 0.5 Macfarland turbidity standardised inoculum of S.aureus from blood agar plate was used .
- Disk required :cefoxitin 30 µg disk.
- Medium used: Mueller –Hinton agar
- QC Recommended: S.aureus ATCC 25923

Test procedure:

The inoculum is swabbed on to the surface of MHA in three dimension and cefoxitin disk is placed and incubated for16-18 hrs at 33 °c- 35 ° c.

Result is interpreted if the zone size $\leq$ 21 mm = *mecA* positive and

$\geq$ 22 mm = *mecA* negative.

Cefoxitin is used as a surrogate marker formec-A mediated oxacillin resistance. Isolates that test as mec A positive shoud be reported as oxacillin [methicillin] resistant strains.

E- test for vancomycin resistance detection :

It is a double ended MIC graded paper E strip [EZY MIC TTM STRIP] contain vancomycin and teicoplanin on a single strip in a concentration gradient manner.The upper half is coated with vancomycin concentration graded tapering

downwards and capable of showing MIC range of 0.5 to 32 µg/ml where as the lower half is coated with teicoplanin drug concentration in reverse direction to show MIC in range of 0.5 to 32 µg/ml .

- Inoculum preparation : from overnight growth on BAP , standardized to 0.5 McFarland
- Medium used : BHI agar plate

Test Procedure:

The BHI plate was streaked 3 times with the inoculum rotating the plate approximately 60ºc each time to ensure an even distribution of inoculums and then the double ended E strip was kept on agar and incubated at35°c for 24 hrs

Interpretation :

Read the MIC value where the edge of inhibition ellipse intersect the side of the E strip.

Organism	**E strip MIC interpretive criteria µg /ml [clsi]**		
	Sensitive	**Intermediate**	**Resistance**
Staphylococcus aureus	≤2	4-8	≥16

ESBL Detection in Escherichia coli,Klebsiella species and Proteus species[119]:

Phenotypic screening methods:

Isolates were subjected to ESBL screening test using cefotaxime (30µg,zone size≤ 27mm) and ceftazidime (30µg, zone size ≤ 22mm) discs.

ESBL Detection by CLSI phenotypic confirmatory method:

Lawn culture of the test isolates was made as for disc diffusion procedure. cefotaxime(30µg), Cefotaxime clavulanic acid disc (30/10µg),and ceftazidime(30µg) Ceftazidime clavulanic acid disc (30/10µg) (Himedia)were placed with in 20-24mm on the surface of the plate.The test isolates were considered to produce ESBL if the zone size around the beta lactamase inhibitor combination disc was increased by ≥ 5 mm than without the beta lactam inhibitor. The test was performed with appropriate controls.

Quality control:

Positive control - K.pneumoniae ATCC 700603

Negative control - E.coli ATCC 25922

IDENTIFICATION OF CANDIDA

Candida isolates from high vaginal swab and urine specimen were identified based on the following identification tests

Growth on SDA:

The colonies appear cream colored ,smooth and pasty.

Gram stain morphology:

Gram positive budding yeast cells with or without pseudohyphae.

Germ tube test :

Germ tube test is used for presumptive identification of Candida albicans. It is a rapid screening test where the production of germ tubes within two hours in contact with the serum is considered as indicative of Candida albicans.

Procedure:

1. The test was done with a fresh growth from a pure culture.
2. A very light suspension of the test organism was inoculated in 0.5ml of the serum (pooled human serum). The optimum inoculums was 10^5-10^6cells per ml.
3. Incubation was done at 37ºC for two hours.
4. One drop of incubated serum was placed on a slide with a cover slip and observed under the microscope for production of Germ tubes.
5. Germ tubes represent initiation of hyphal growth, arising directly from the yeast cell. They have parallel walls at their point of origin and are not constricted.
6. A positive record was taken if 30% of cells showed germ tube production.

Morphology on CHROM agar Medium [130]:

It is selective and differential type of chromogenic medium, which is useful for identification of various Candida species . Due to chromogenic substrate in the medium, the colony morphology and color have been well defined when it is used to isolate the yeasts. The CHROM agar candida shows following colors of colonies after incubation at 30ºc for a period of 48 to 72 hrs:

C.albicans : Light green , C.dubliniensis :Dark green ,C.glabrata :Pink to purple , C.krusei :Pink , C.parapsilosis :Cream to pale pink , C.tropicalis :Blue with pink halo.

Sugar fermentation reactions for Candida species[130]:

A liquid medium containing peptone (1%),sodium chloride (0.5%),Andrade's indicator(0.005%) was prepared and sterilised by autoclaving at 120 ^{o}c for 15 min at 15 pounds pressure .Filter sterilised sugar was added at a concentration of 2% to the medium. It was poured into sterile test tubes along with Durham's tube. Each broth was inoculated with 0.1ml of heavy inoculums of yeast grown on sugar free media. The tubes were incubated at 25 ^{o}c upto 1 week. Every 48 hrs the tubes were examined for the production of acid (yellow color) and gas (in Durham's tube).Production of acid and gas in tube was taken as positive test.

SUGAR FERMENTATION TEST:

Candida species	Glucose	Maltose	Sucrose	Lactose
C.albicans	AG	AG	-	-
C.troplicalis	AG	AG	AG	-
C.kefyer	AG	AG	AG	-
C.guilliermondii	AG	-	AG	-
C.parapsilosis	AG	-	-	-
C.krusei	AG	-	-	-
C.glabrata	AG	-	-	-

A=Acid production , G=Gas production

Sugar assimilation test for Candida species[130]:

Based on the utilization of different carbohydrates Candida species can be identified.

Three generation of the test organism is done on nutrient agar slope. Add 2ml saline to 3rd generation slope, wash and make suspension. Transfer 2ml of yeast suspension into another sterile test tube containing 1.5ml of yeast nitrogen base (YNB). Add this into 13.5ml of molten and cooled (40-45 ºC) plain agar. Twirl and pour into sterile petri dish which is prelabelled with 20 % sugars and allow it to solidify . Place the disc containing sugars in the appropriately marked area and incubate at 25 ºc. Growth around the disc was taken as assimilation of the sugar.

SUGAR ASSIMILATION TEST:

Candida spp.	Glu	Mal	Suc	Lac	Cel	Gal	Tre	Raf	Mel	Xyl	Ino	Dul
C.albicans	+	+	+	+	+	+	+	-	-	+	-	-
C.tropicalis	+	+	+	-	+	+	+	-	-	+	-	-
C.kefyer	+	-	+	+	+	+	-	+	-	+	-	-
C.parapsilosis	+	+	+	-	-	+	+	-	-	+	-	-
C.guilliermondii	+	+	+	-	+	+	+	+	+	+	-	+
C.krusei	+	-	-	-	-	-	-	-	-	+	-	-
C.glabrata	+	-	-	-	-	-	+	-	-	+	-	+

Note:Glu=Glucose, Mal=Maltose, Suc=Sucrose, Lac=Lactose, Cel=Cellobiose, Gal=Galactose, Tre=Trehalose, Raf=Raffinose, Mel=Melibiose, Xyl=Xylose, Ino=Inositol, Dul=Dulcitol , +=positive reaction ,- = negative reaction.

METHOD FOR ANTIFUNGAL DISK DIFFUSION SUSCEPTIBILITY TESTING FOR YEAST(CLSI M44-A2)

REAGENTS THAT WERE USED FOR DISK DIFFUSION TEST

Mueller Hinton Agar +2% glucose and 0.5µg/ml Methylene blue dye(GMB)medium.

PROCEDURE FOR PERFORMING THE DISK DIFFUSION TEST

Steps for preparation of the inoculums are as follows:

1. All organisms were subcultured on Sabourauds dextrose agar.
2. Inoculum was prepared by picking up five distinct colonies and suspended in 5ml of sterile saline.
3. The resulting suspension was matched with 0.5 McFarland standard at 530nm wavelength.

Inoculation of test plates

1. After 15 mins of suspension preparation a swab is dipped into the suspension and plated onto the dried surface of Mueller Hinton + GMB agar plates
2. The plate was streaked 3 times rotating the plate approximately 60ºc each time to ensure an even distribution of inoculums
3. Drug impregnated disks were applied on the plate after 15 mins.
4. The plates were read after 24 hrs of incubation.

Zone Diameter Interpretative Standards

Susceptible (S):

The susceptibility category implies that an infection an infection due to the strain can be appropriately treated with the dose of antimicrobial agent recommended for that type of infection .

Susceptible –Dose Dependent(SDD):

The Susceptible dose dependent category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolate.

Resistant(R):

The resistant strains are those that are not inhibited by the usually achievable concentration of the agent with normal dosage schedules.

Zone diameter interpretive standards for Candida species

Antifungal agent	**Disc content**	**Zone diameter interpretation**		
		R	**SDD**	**S**
Fluconazole	25μg	≤14	15-18	≥19
Voriconazole	1μg	≤13	14-16	≥17

Results

RESULTS

A total of 200 antenatal patients were included in this study out of which 33 patients were from out patient department and others were from inpatient department of Institute of Obstetrics and gynaecology, Egmore. The study was done between March 2017 to February 2018 . The results obtained from the study are as follows.

TABLE 1:DISTRIBUTION OF AGE (IN YEARS) OF PATIENTS INCLUDED IN THE STUDY (n=200)

AGE IN YEARS	NO OF PATIENTS	PERCENTAGE
11-15	2	0.8 %
16-20	22	8.3 %
21-25	89	33.5%
26-30	59	22.2%
31-35	21	7.9%
36-40	6	2.3%
41-45	1	0.4%
TOTAL	200	100%

The above table shows distribution of age in years of the antenatal patients who were included in the study.The maximum number of patients were in the age group between 21-25 years (33.5%) followed by 26-30 years (22.2%).

FIGURE 1

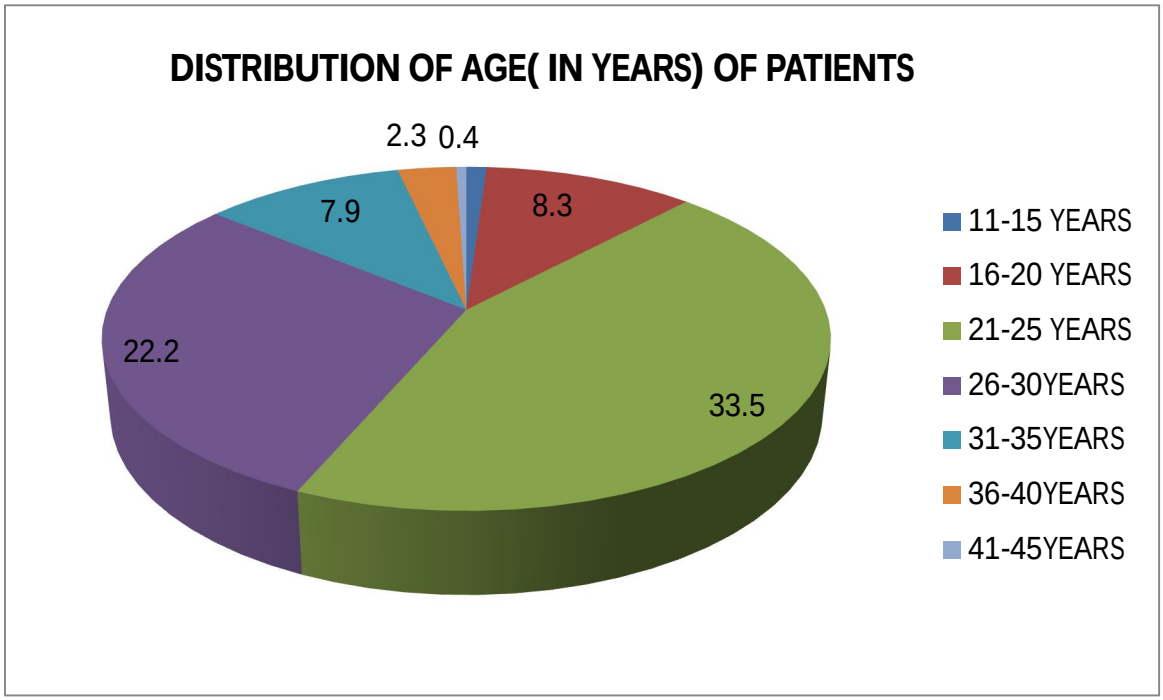


TABLE 2:DISTRIBUTION OF PRESENTING COMPLAINTS OF THE PATIENTS

SYMPTOMS	NO OF PATIENTS	PERCENTAGE
DISCHARGE	155	77.5%
FOUL SMELL	30	15%
LOWER ABDOMINAL PAIN	65	32.5%
BURNING MICTURITION	42	21%
VAGINAL DISCOMFORT	25	12.5%

FIGURE 2

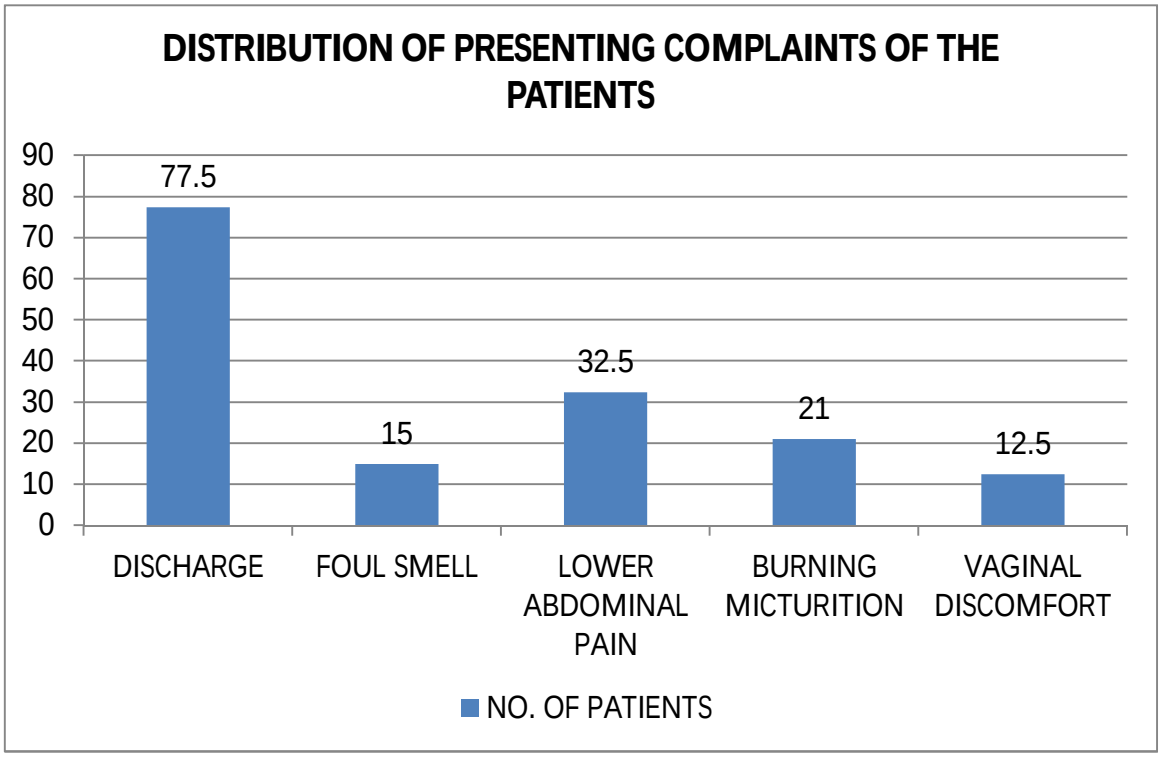


TABLE 3 :AGE WISE DISTRIBUTION OF THE PRESENTING COMPLAINTS

AGE GROUP	11-15 years	16-20 Years	21-25 years	26-30 years	31-35 years	36-40 years	41-45 years	TOTAL	%
DISCHARGE	2 (1.3%)	16 (10.3%)	64 (41.3%)	49 (31.6%)	19 (12.3%)	4 (2.6%)	1 (0.6%)	155	77.5
FOUL SMELL	0	3 (10%)	12 (40%)	10 (33.3%)	3 (10%)	1 (3.3%)	1 (3.3%)	30	15
LOWER ABDOMINAL PAIN	0	9 (13.8%)	32 (49.2%)	18 (27.7%)	3 (4.6%)	3 (4.6%)	0	65	32.5
BURNING MICTURITION	0	2 (4.8%)	20 (47.6%)	16 (38.1%)	2 (4.8%)	2 (4.8%)	0	42	21
VAGINAL DISCOMFORT	0	5 (20%)	9 (36%)	8 (32%)	2 (8%)	1 (4%)	0	25	12.5

The above table shows that the commonest symptom that was presented by the patients included in the study was vaginal discharge. Vaginal discharge was the predominant complaint in 77.5% of the total patients and in 41.3% of patients in 21-25 years of age. The next common complaint presented was lower abdominal pain which was seen in 32.5% of patients and in 49.2% of patients in 21-25 years of age. Other symptoms such as burning micturition (21%),foul smell (15%) and vaginal discomfort were also presented by the patients.

TABLE 4 :TRIMESTER WISE DISTRIBUTION OF INFECTIOUS VAGINITIS

TRIMESTER	TOTAL PATIENTS	BV	VVC	TV	POSITIVE	Statistic Chi-square test
1	32(16%)	6(14.3%)	3(16%)	0	9 (13.8%)	p-value >0.05
2	99(49.5%)	19(45.2%)	12(49.5%)	0	31(47.7%)	
3	69(34.5%)	17(24.6%)	8(34.5%)	0	25(38.5%)	
Total	200	42	23	0	65	

The higher rate of vaginal infection was found in 2nd trimester of pregnancy 31(47.7%) followed by 3rd trimester 25(38.5%) and then 1st trimester 9(13.8%). However the difference was not statistically significant. (p>0.05)

TABLE 5 :TRIMESTER WISE DISTRIBUTION OF UTI

TRIMESTER	TOTAL PATIENTS	UTI	Statistic Chi-square test
1	32(16%)	1(3.1%)	P-value<0.05
2	99(49.5%)	15(46.9%)	
3	69(34.5%)	16(50%)	
Total	200	32(16%)	

The higher rate of urinary tract infection was found in 3rd trimester of pregnancy 16(50%) followed by 2nd trimester 15(46.9%) and then 1st trimester 1(3.1%).The difference was statistically significant. (p<0.05)

FIGURE 3

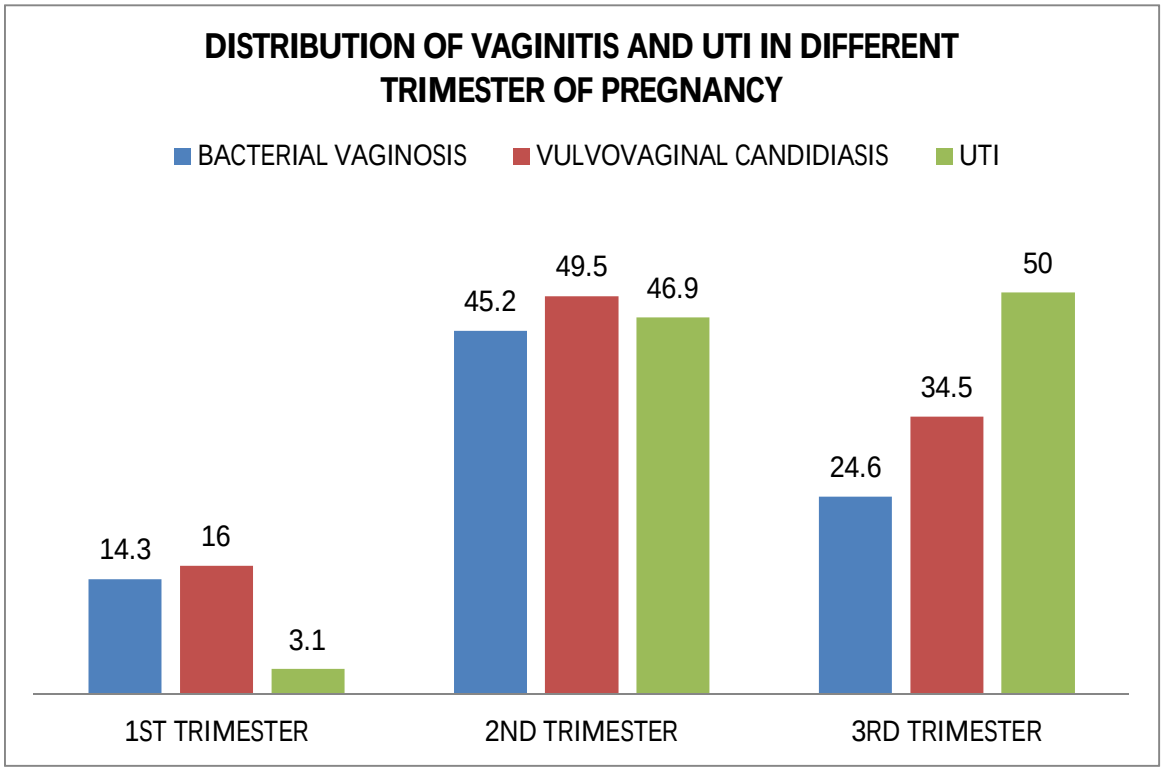


TABLE 6 :AGE WISE DISTRIBUTION OF VAGINITIS AMONG ANTENATAL WOMEN (n=200)

AGE CATEGORY	TOTAL PATIENTS	INFECTION			POSITIVE	Statistic Chi-square test
		BV	VVC	BV+VVC		
11-15	2	0	0	0	0 (0%)	P-value>0.05
16-20	22	3 (7.1%)	2 (8.7%)	1 (14.3%)	5 (7.6%)	
21-25	89	18 (42.9%)	8 (34.8%)	2 (28.6%)	26 (40%)	
26-30	59	18 (42.9%)	10 (43.5%)	2 (28.6%)	28 (43.1%)	
31-35	21	2 (4.8%)	2 (8.7%)	1 (14.3%)	4 (6.1%)	
36-40	6	1 (2.4%)	1 (4.3%)	1 (14.3%)	2 (3.2%)	
41-45	1	0	0	0	0	
TOTAL	200	42	23	7	65	

The prevalence of infectious vaginitis in the study was found to be 32.5%.The infection rate was high among the age group 26-30 years (43.1%) and least infected group was 11-15 and 41-45 years (0.0%).Mixed infection of BV and VVC was found in age group 21-25 and 26-30 years. Both BV and VVC were found high in age group 26-30 years. However the difference was not statistically significant($p>0.05$).

TABLE 7 :AGE WISE DISTRIBUTION OF UTI AMONG ANTENATAL WOMEN(n=200)

AGE CATEGORY	TOTAL PATIENTS	UTI	Statistic Chi-square test
11-15	2	0 (0.0%)	P-value<0.05
16-20	22	2 (6.2%)	
21-25	89	11(34.4%)	
26-30	59	17(53.1%)	
31-35	21	0(0.0%)	
36-40	6	1(3.1%)	
41-45	1	1(3.1%)	
TOTAL	200	32(16%)	

The UTI was predominantly seen in antenatal patients with age group 26-30 years followed by age group 21-25 years . These two age groups had maximum number of patients included in the study.

FIGURE 4

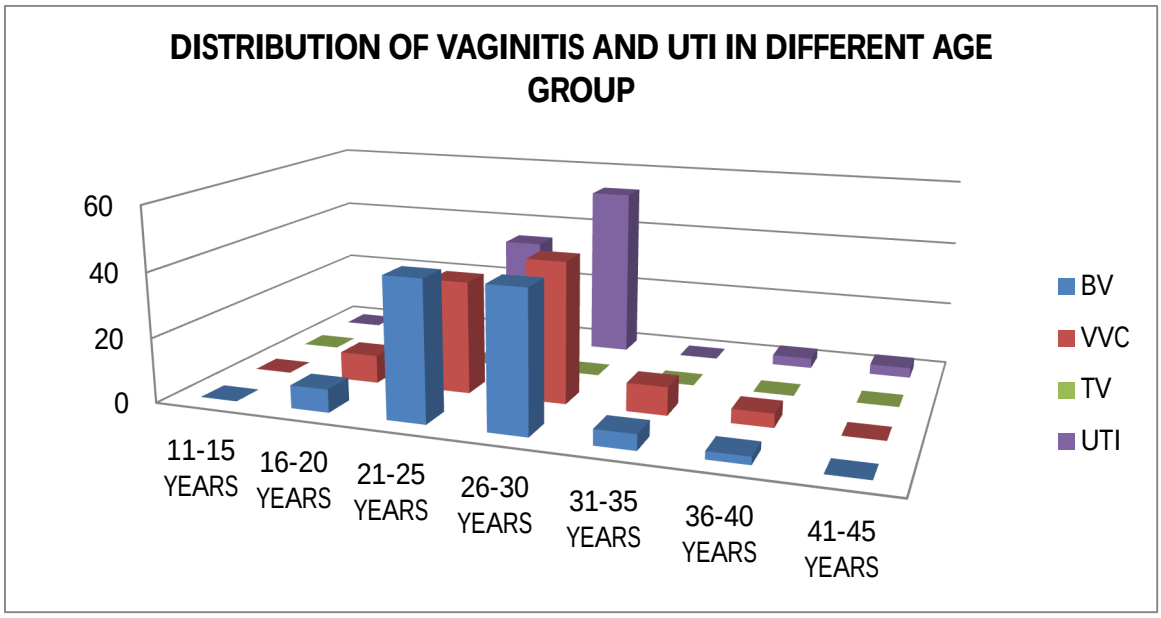


TABLE 8 :ASSOCIATION OF BACTERIAL VAGINOSIS (BV) WITH UTI DURING PREGNANCY(n=200)

DIAGNOSIS	UTI PRESENT	UTI ABSENT	TOTAL	Statistic Chi-square test
BV PRESENT	15	27	42	P<0.05
BV ABSENT	17	141	158	
TOTAL	32	168	200	

n=200	NO.	%
BACTERIAL VAGINOSIS PRESENT	42	21
BACTERIAL VAGINOSIS ABSENT	158	79
URINARY TRACT INFECTION PRESENT	32	16
URINARY TRACT INFECTION ABSENT	168	84
BACTERIAL VAGINOSIS ASSOCIATED WITH UTI	15	35.7
UTI WITHOUT BV	17	10.7

The above table shows that BV was present in 42 antenatal patients (21%) and UTI was present in 32 patients (16%). About 15 patients with BV(out of 42) had associated UTI. The association between BV and UTI was found to be statistically significant($p<0.05$).

TABLE 9 :ASSOCIATION OF

VULVO VAGINAL CANDIDIASIS (VVC) WITH UTI

DIAGNOSIS	UTI PRESENT	UTI ABSENT	TOTAL	Statistic Chi-square test
VVC PRESENT	4	19	23(11.5%)	P>0.05
VVC ABSENT	28	149	177(88.5%)	
TOTAL	32	168	200	

The above table shows that VVC was present in 23 antenatal patients (11.5%) and UTI was present in 32 patients (16%). About 4 patients with VVC(out of 23) had associated UTI. VVC associated with UTI was seen in 17.4% of the patients. However the association between VVC and UTI was statistically not significant($p>0.05$).

TABLE 10 :AMSEL'S CRITERIA FOR DIAGNOSIS OF BV

S.NO	VARIABLE	PRESENT
1.	VAGINAL DISCHARGE	155(77.5%)
2.	CLUE CELLS	46(23%)
3.	WHIFF TEST	70(35%)
4.	pH > 4.5	132(66%)

Based on Amsel's criteria ,35 cases were labelled to have BV. The predominant variable present was vaginal discharge 155(77.5%) and least occurring variable in patients with BV was presence of clue cells46(23%) in vaginal wet mount.

FIGURE 5

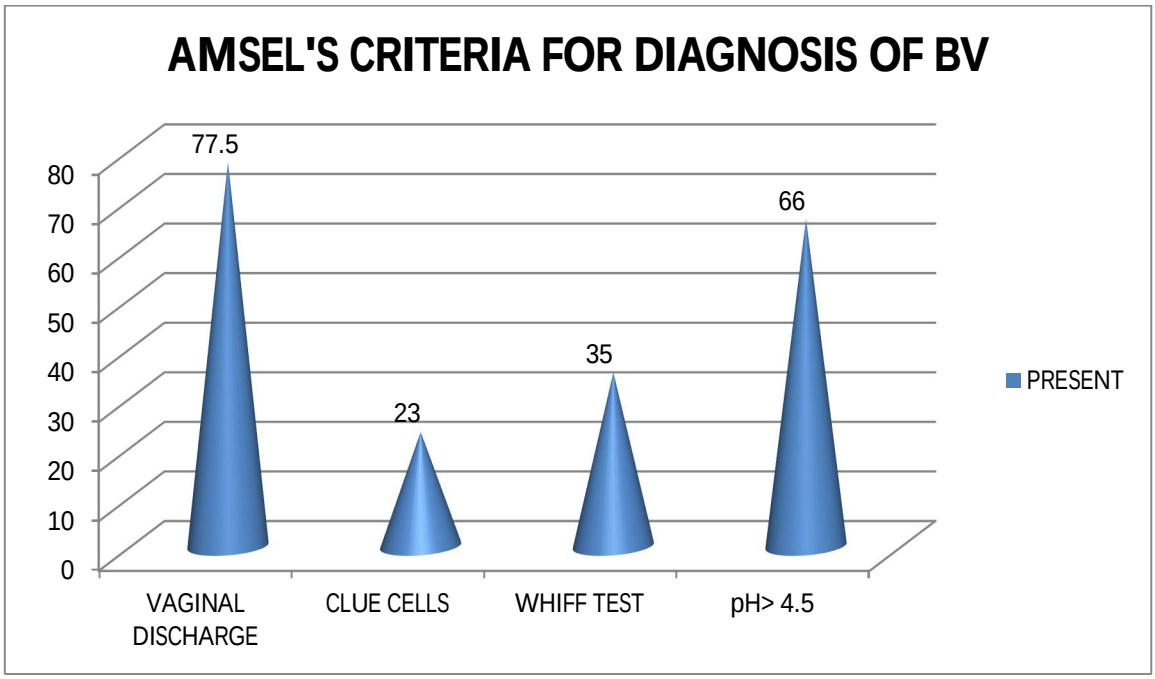


TABLE 11 :NUGENT'S SCORING FOR DIAGNOSIS OF BV

S.NO	SCORE	NO. OF CASES
1.	0-3	85(42.5%)
2.	4-6	73(36.5%)
3.	7-10	42(21%)
	TOTAL	200

Based on Nugent's criteria, 42(21 %) cases were labelled to have BV.

FIGURE 6

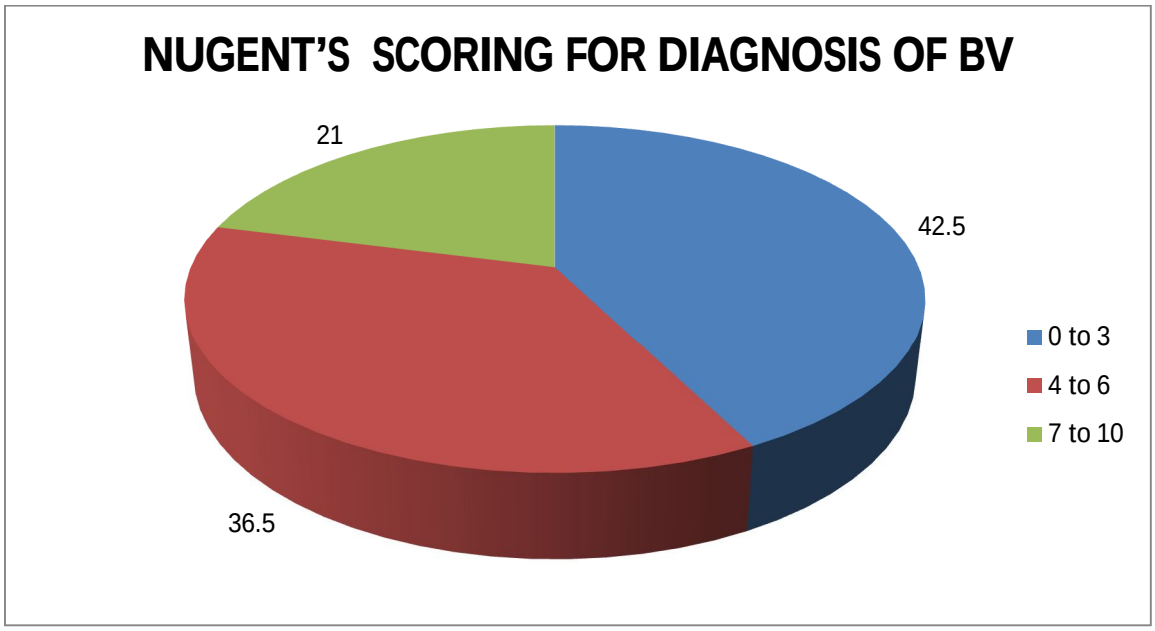


TABLE 12 :DIAGNOSIS OF BACTERIAL VAGINOSIS BY COMPARISON BETWEEN AMSEL'S CRITERIA AND NUGENT'S SCORING.

METHODS OF DIAGNOSIS		DIAGNOSIS OF BV BY NUGENTS SCORING			p-value
		Score >7 BV PRESENT (n=42)	Score 0-6 BV ABSENT (n=158)	Total (n=200)	
AMSEL'S CRITERIA	BV PRESENT	28	7	35	p<0.05
	BV ABSENT	14	151	165	

In comparison with gold standard Nugent's criteria ,the sensitivity ,specificity, positive predictive value and negative predictive value of Amsel's criteria were 80%, 91.5%, 66.7% and 95.6% respectively. Statistical analysis showed that both methods could be used as a means for diagnosis of Bacterial vaginosis ($p < 0.05$).

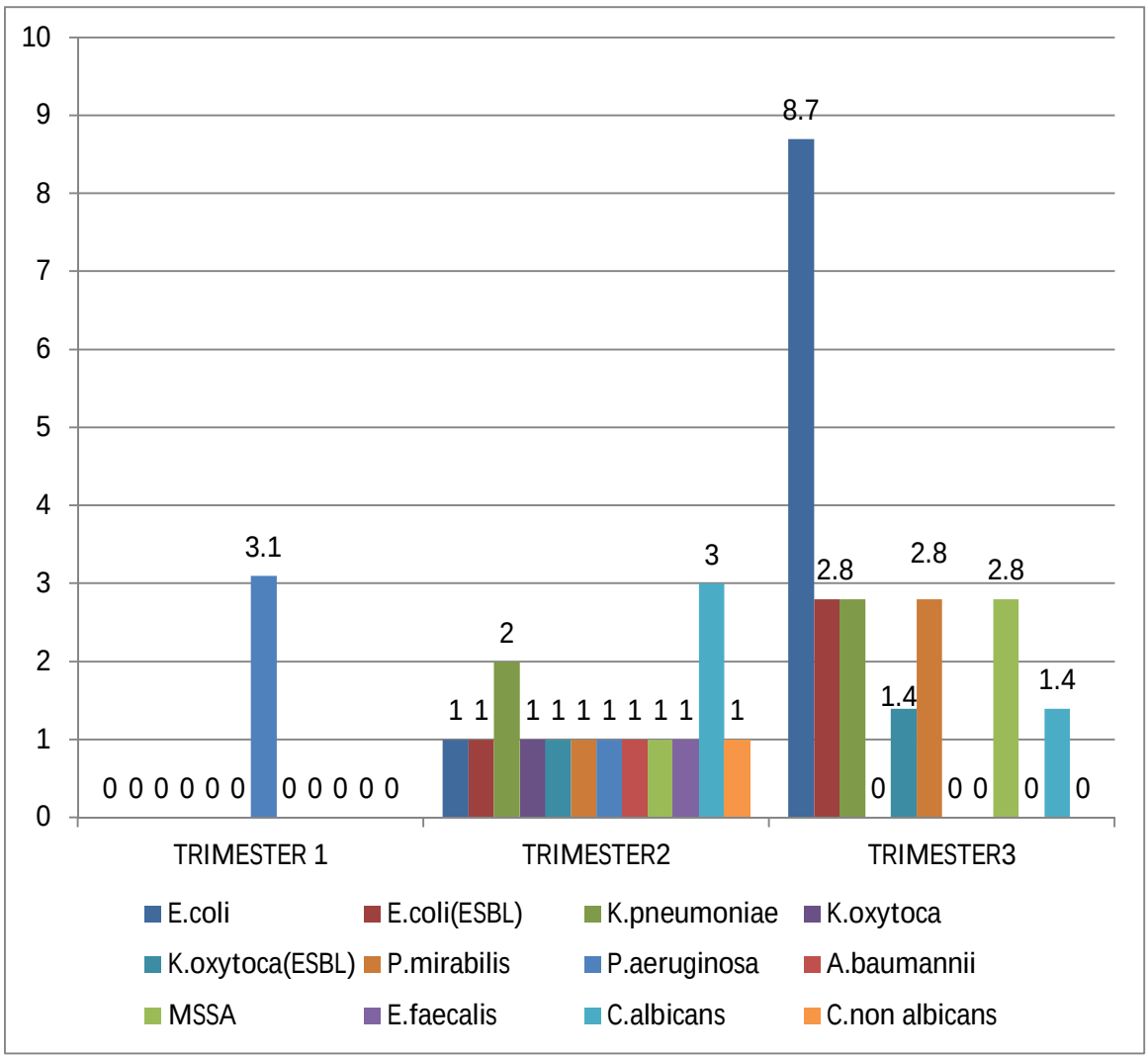
TABLE 13 :BACTERIAL ISOLATES DETECTED FROM HVS AND URINE CULTURE

ISOLATES FROM HVS	TOTAL	MDR(%)	ISOLATES FROM URINE	TOTAL	MDR(%)
E.coli	13	3 (23.0)	E.coli	10	5(50.0)
K.pneumoniae	7	1 (14.2)	K.pneumoniae	4	2(50.0)
K.oxytoca	7	1 (14.2)	K.oxytoca	3	0(0.0)
P.mirabilis	3	0 (0.0)	P.mirabilis	3	0(0.0)
P.vulgaris	0	0(0.0)	P.aeruginosa	2	0(0.0)
S.aureus	7	2(28.5)	S.aureus	3	1(33.3)
S.epidermidis	2	0(0.0)	E.faecalis	1	0(0.0)
S.agalactiae	1	0(0.0)	A.baumannii	1	1(100.0)
TOTAL	40	7	TOTAL	27	9

The predominant bacteria isolated from both HVS and urine was E.coli. Of the isolated bacteria from HVS 28.5% of S.aureus ,23% of E.coli , 14.2% of K.pneumoniae and K.oxytoca were Multidrug resistant (MDR) isolates. Also 50% of E.coli, 50% K.pneumoniae ,33.3% of S.aureus isolated from urine were MDR isolates. An isolate of A.baumannii from urine specimen was also MDR.

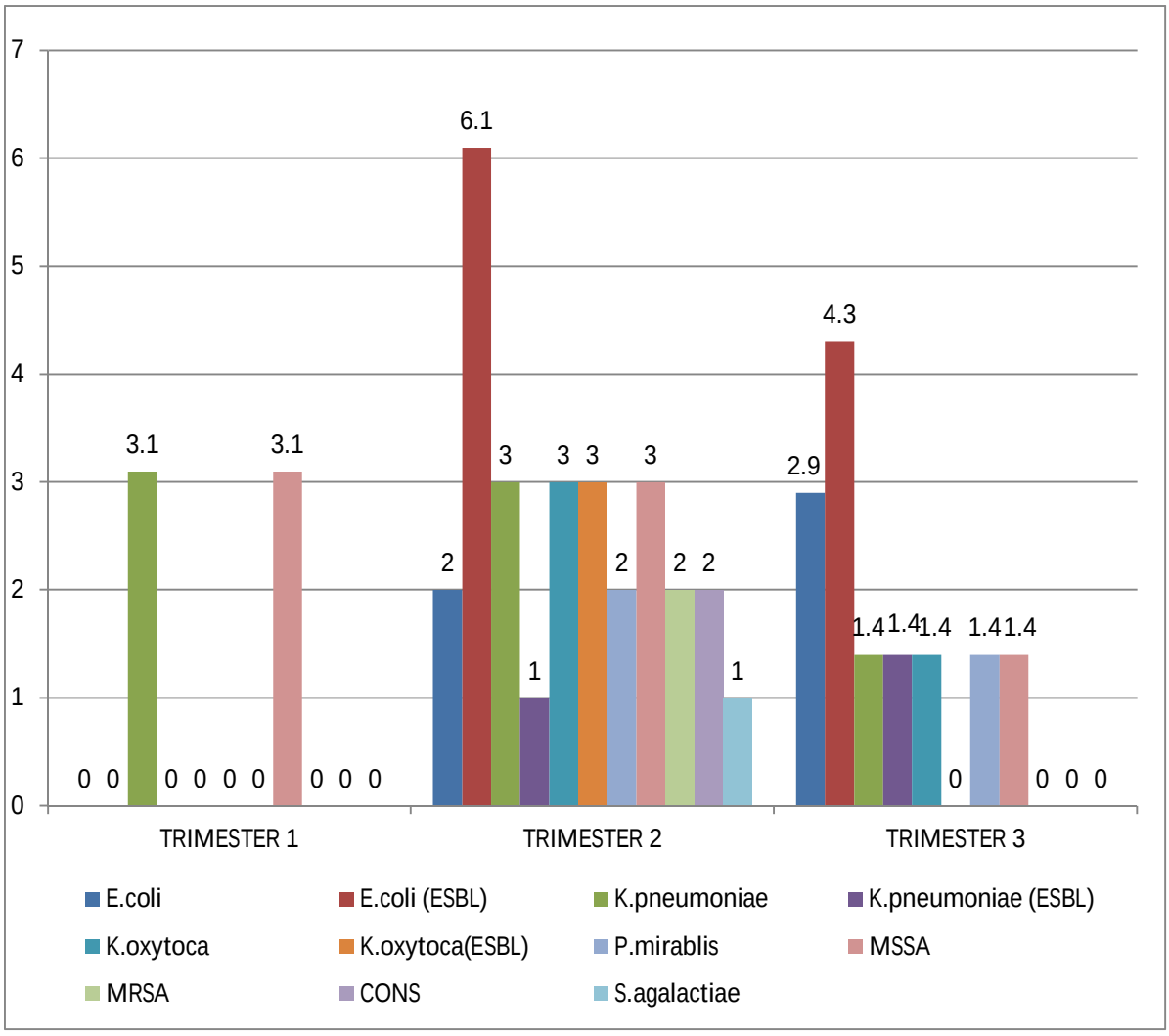
FIGURE 7 :TRIMESTER WISE DISTRIBUTION

OF ISOLATES FROM URINE



About 2.8% of E.coli and 1.4% K.oxytoca isolates in 2nd trimester were found to be ESBL producers. Candida albicans (3%) was found more common in 2nd trimester UTI. Overall higher rate of UTI was found in 3rd trimester of pregnancy and the difference was statistically significant ($p<0.05$)

FIGURE 8 :TRIMESTER WISE DISTRIBUTION OF ISOLATES FROM HVS



The 2nd trimester of pregnancy showed maximum bacterial growth of 28.3% followed by 3rd trimester 14.5%. however the difference was not statistically significant($p>0.05$)

TABLE 14 :ANTIMICROBIAL SUSCEPTIBILITY PATTERN FOR URINARYISOLATES GNB:

DRUGS / ISOLATES	GM 10µg	CTX 30µg	CEC 30/ 10µg	NIT 300µg	NOR 5µg	CAZ 30µg	PT 100/ 10µg	CIP 5µg	COT 1.25 /23.75 Mg	AK 30µg	TET 30µg	IMP 10µg
	%	%	%	%	%	%	%	%	%	%	%	%
E.coli n=10	60	30	80	80	60	-	60	-	-	50	100	100
K.pneumoniae n=4	75	25	75	50	50	-	50	-	-	50	100	100
K.oxytoca n=3	100	33	100	100	100	-	-	-	-	100	-	-
P.mirabilis n=3	100	100	100	*	100	-	100	33	100	-	*	-
P.aeruginosa n=2	100	*	#	#	0	100	100	0	*	-	*	100
A.baumannii n=1	0	-	#	-	-	0	100	0	0	-	0	0

NOTE: *** :**Intrinsic resistance , **- :** Not tested , **#:** Not applicable

TABLE 15 :ANTIMICROBIAL SUSCEPTIBILITY PATTERN FOR URINARY ISOLATES GPC:

DRUGS / ISOLATES	PEN 10U (%)	NOR 5µg (%)	NIT 300µg (%)	COT 1.25/ 23.75 µg (%)	CIP 5µg (%)	CX 30µg (%)	ERY 15µg (%)	TET 30µg (%)	HLG 120µg (%)
S.aureus n=3	33	-	66.6	66.6	66.6	66.6	66.6	100	#
E.faecalis n=1	100	100	100	*	0	#	0	100	100

NOTE: ***:** Intrinsic resistance , **- :** Not tested , **#:** Not applicable

Among the urinary isolates cefotaxime was found to be 70% resistant toE.coli, 75%resistant K.pneumoniae and 67% resistant to K.oxytoca. cefotaximeclavulanate was found to be 20% resistant to E.coli, and 25% resistant to K.pneumoniae , 100% sensitive to K.oxytoca and P.mirabilis. Higher susceptibility was seen with tetracycline(100%) for E.coli, K.pneumoniae and E.faecalis. Imipenem100% sensitive for E.coli and K.pneumoniae . P. mirabilis was 100% sensitive to piperacillin tazobactum ,cefotaxime,cotrimoxazole, gentamicin and norfloxacin . P.aeruginosa was 100% sensitive to gentamicin ,ceftazidime and piperacillin tazobactum. A.baumannii was MDR but 100% sensitive to piperacillin tazobactum.

TABLE 16 :ANTIMICROBIAL SUSCEPTIBILITY PATTERN FOR VAGINAL ISOLATES GNB:

DRUGS / ISOLATES	GM 10µg (%)	CTX 30µg (%)	CEC 30 /10µg (%)	PT 100/ 10µg (%)	CIP 5µg (%)	COT 1.25/ 23.75 µg (%)	AK 30µg (%)	TET 30µg (%)	IMP 10µg (%)
E.coli n=13	90	36	86	-	78.5	57	43	100	100
K.pneumoniae n=7	100	57	85.7	-	100	85.7	85.7	100	100
K.oxytoca n=7	85.7	42.8	71	-	57	57	71	100	100
P.mirabilis n=3	100	100	100	100	100	0	100	*	-

NOTE:* :Intrinsic resistance **- :** Not tested

TABLE 17 :ANTIMICROBIAL SUSCEPTIBILITY PATTERN FOR VAGINALISOLATES GPC:

DRUGS / ISOLATES	PEN 10U (%)	COT 1.25/ 23.75 µg (%)	CIP 5µg (%)	CX 30µg (%)	CTX 30µg (%)	ERY 15µg (%)	TET 30µg (%)	VAN 30µg (%)	CK 30µg (%)	LZ 30µg (%)
S.aureus n=7	43	57	71	71	#	71	71	#	100	100
S.epidermidis n=2	0	100	0	100	#	100	100	#	-	-
S.agalactiae n=1	0	0	#	#	100	100	-	100	100	-

NOTE: - : Not tested , **#:** Not applicable

Organism	E strip MIC for vancomycin	
	MIC	REPORT
Staphylococcus aureus (MRSA) n=2	<2	Sensitive(100%)

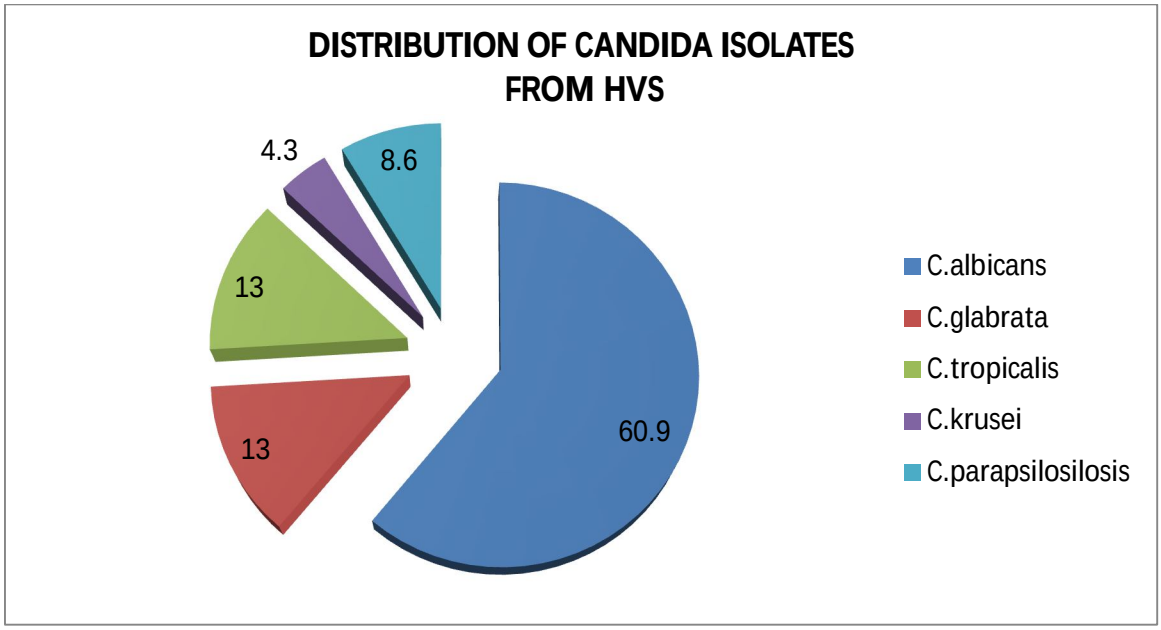
Among the vaginal isolates Cefotaxime was found to be 64% resistant to E.coli, 43% resistant K.pneumoniae and 57% resistant to K.oxytoca. Cefotaxime clavulanate was found to be 14% resistant to E.coli, and K.pneumoniae and 29% resistant to K.oxytoca. Higher susceptibility was seen with Tetracycline(100%) for E.coli, Klebsiella spp and S.epidermidis. Imipenem100% sensitive for E.coli and Klebsiella spp . P. mirabilis was 100% sensitive to Piperacillin tazobactum, ciprofloxacin, cefotaxime, amikacin and gentamicin .

S.aureus was highly susceptible to chloramphenicol (100%) and linezolid(100%) and least sensitive to penicillin. Two MRSA isolates had vancomycin MIC in susceptible range. S.agalactiae was 100% sensitive to erythromycin ,cefotaxime ,chloramphenicol and vancomycin.

TABLE 18 :CANDIDA SPECIES ISOLATED FROM HVS

CANDIDA SPECIES	NO. OF ISOLATES n =23	%
C.albicans	14	60.9
C.glabrata	3	13
C.tropicalis	3	13
C.krusei	1	4.3
C.parapsilosis	2	8.6

FIGURE 9



Candida albicans(60.9%) was the commonest species isolated in this study. Of the non-albicans species isolated C.glabrata(13%) and C.tropicalis (13%) were the most common.

TABLE 19 :ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA ISOLATES FROM HVS SAMPLES

ISOLATES / DRUGS	C.albicans n=14		C.glabrata n=3		C.troplicalis n=3		C.parapsilosis n=2	
Fluconazole (25 μg)	14	100%	1	33%	3	100%	2	100%
Itraconazole (10 μg)	14	100%	1	33%	3	100%	2	100%
Voriconazole (1 μg)	13	93%	0	0%	3	100%	2	100%
Nystatin B (1 μg)	12	86%	2	67%	2	100%	2	100%

DRUG / ISOLATE	Nystatin B susceptibility (1 μg)
C.krusei (n=1)	**100%**

***C.krusei is inherently resistant to azoles so tested only with Nystatin B**

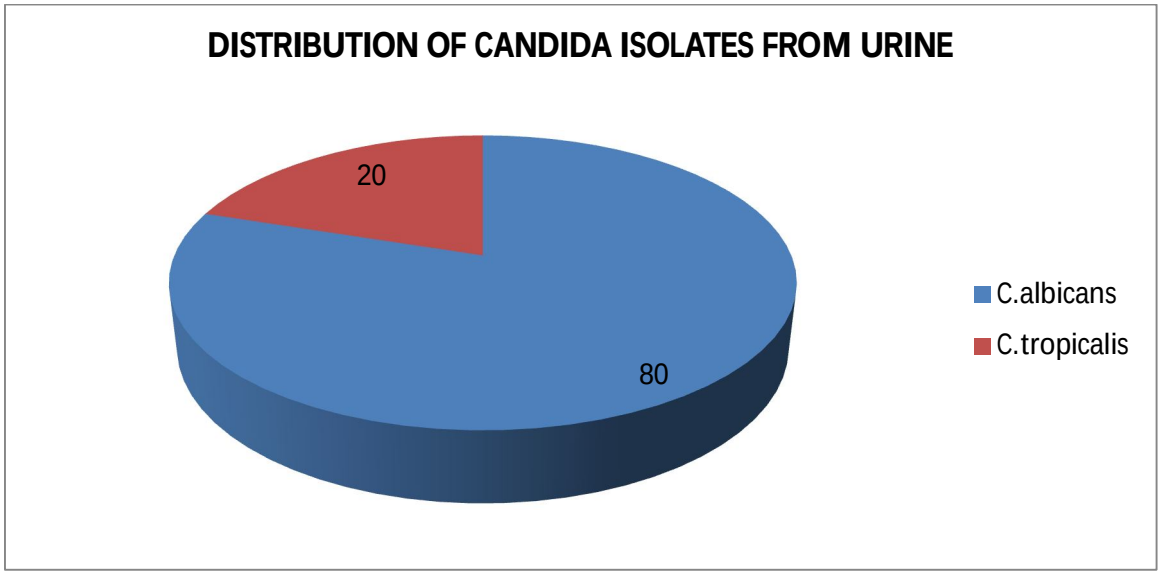
Candida albicans isolates were 100% sensitive to Fluconazole ,Itraconazole and least sensitive to Nystatin B.

Among Candida non albicans species C.tropicalis and C.parapsilosis isolates were 100% sensitive to fluconazole ,itraconazole , voriconazole and nystatin B. C.glabrata was only 33% sensitive to fluconazole ,itraconazole and C.krusei was100% sensitive to nystatin B.

TABLE 20 :CANDIDA SPECIES ISOLATED FROM URINE :

CANDIDA SPECIES	NO. OF ISOLATES n =5	%
C.albicans	4	80
C.tropicalis	1	20

FIGURE 10



Candida albicans(80%) was the commonest species isolated . Only one non-albicans species was isolated which was identified as C.tropicalis (20%).

TABLE 21 :ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA ISOLATES FROM URINE SAMPLES

ISOLATES / DRUGS	C.albicans n=4		C.troplicalis n=1	
Fluconazole (25 μg)	4	100%	1	100%
Itraconazole (10 μg)	4	100%	1	100%
Voriconazole (1 μg)	4	100%	1	100%
Nystatin B (1 μg)	4	100%	1	100%

Candida albicans isolates were 100% sensitive to Fluconazole ,Itraconazole and Nystatin B and 75% sensitive to voriconazole.

C.tropicalis was 100% sensitive to fluconazole ,itraconazole , voriconazole and nystatin B.

Candida albicans was the commonest species among Candida isolated from HVS(60.9%) and urine (80%) . C.tropicalis(20%) was the only Candida non albicans species isolated from urine specimen and it was also found in HVS (13%). C.tropicalis both from HVS and urine was 100% sensitive to all azoles tested and nystatin B. Whereas Candida non albicans species inherently resistant to azoles (C.krusei) was isolated from HVS culture.

Discussion

DISCUSSION

This study was conducted in the antenatal patients irrespective of their gestational age attending the Institute of obstetrics and Gynaecology. About 200 patients who fulfilled the inclusion criteria were included in the study.

The prevalence of infectious vaginitis among antenatal women was found to be 32.5%. Similar reports were obtained in study done by Lamichnane et al[18] in Nepal on prevalance of infectious vaginitis which was found to be 40%. But another study by O.A.Olowe et al[20] in south western Nigeria reported prevalence of vaginitis to be 76% which was much higher when compared to our study.

Vaginitis was most common in the age group 26-30years(43.1%) followed by 21-25years(40%) which might be because these age groups represent reproductively active age, having high sexual exposure .The study by Dipak bhargava et al[120] conducted in Nepal showed similar results of infectious vaginitis being most common in the age group 20-29years (56.1%).However in both of these studies the age of the patients in the reproductive age group does not reveal any significant relationship with vaginitis ($p>0.05$).

In our study higher rate of vaginal infection was found in 2nd trimester of pregnancy (47.7%). In contrast to our result study by O.A Adeyeba et al[1] in Nigeria had higher rate of vaginal infection during 3rd trimester of pregnancy but

reported that gestational age has no statistical significance in distribution of vaginal infection ($p>0.05$) which was similar to our study.

Bacterial vaginosis was the most prevalent type of vaginitis (21%) followed by vulvovaginal candidiasis(11.5%) and mixed infection was seen in only 3.5% of the patients. In concordance with our finding study by Lata et al [52]in Lucknow showed BV in 20.5% and mixed infection in 1.5% of antenatal patients but in contrast VVC was seen in only 4.5% of the patients.

For most of the bacterial diseases culture is the gold standard method for diagnosis ; however, culture cannot become the gold standard for diagnosis of BV as the organisms which are involved in BV cannot be easily isolated in the laboratory and as normal women may also have this flora in their vagina in small numbers . Alternative diagnostic methods have been developed, such as the polymerase chain reaction (PCR), rapid nucleic acid hybridization test and proline amino peptidase activity for the diagnosis of BV. However, most of these are expensive and their sensitivities and specificities do not offer a huge advantage over the classical methods.

As the prevalence of BV in developing countries like ours are high, countries with limited resources have a great need for inexpensive diagnostic methods that are reliable and unifies clinical and microbiological parameters to make it more sensitive while retaining its specificity.

Amsel's and Nugent's scoring remain the most practical, viable and economical options for diagnosing bacterial vaginosis, especially in developing countries [21,35].Therefore in this study diagnosis of BV was done based on Amsel's clinical criteria and Nugent's scoring of vaginal smears. Amsel's criteria detected 35/200(17.5 %) whereas Nugent's score identified 42/200 (21%)patients as having BV as shown in Table 12. In comparison with Nugent's criteria, the sensitivity, specificity, positive predictive value and negative predictive value of Amsel's criteria were 80%, 91.5%, 66.7% and 95.6% respectively. Statistical analysis showed that both methods (Amsel's and Nugent's criteria) could be used as a means for the diagnosis of bacterial vaginosis ($p<0.05$) .Study by Rajeshwar Rao et al[81] al in Hyderabad and Nawani et al[121] showed similar findings to our results.

Vaginal discharge was the predominant complaint and the common criteria which were present in almost 77.5% of the patients included in the study.This was found to be in correlation with the study conducted by A.Agarwal et al[126]., who showed a prevalence of 84% in his study. pH was found to be greater than 4.5 in about 66% of patients in this study which was lesser compared to results by A.Agarwal et al.,(88%).Amine test was found to be positive in around 35% of patients in this study compared to 68% of patients in the above study.Clue cells were also found to be much lower (23%) in our study when compared to the above study(64%). A significant correlation was seen between bacterial vaginosis

and the occurrence of symptoms like foul smelling discharge,lower abdominal pain and burning micturition($p<0.05$)

In this study, the overall prevalence of Trichomonas vaginalis(TV) was 0 % as shown in table 4. This suggest the non-existence of relatively high rate of this infection among pregnant women attending Institute of obstetrics and Gynaecology, Egmore which could be due to improved healthy living. Similarly study done on prevalence of TV among pregnant women attending Irrua Specialist Teaching Hospital ,Nigeria by Ochei Kingsley Chinedum et al[122] showed 0% prevalence and study by Lata et al [52] in Lucknow also reported 0% prevalence of TV . However Nourian A et al[123] reported 3.3% prevalence of TV among pregnant population in North west Iran.

Table 5 shows the prevalence of UTI was found to be 16% with higher rate of infection found in 3rd trimester (50%) followed by 2nd trimester (46.9%) and the difference was found to be statistically significant($p<0.05$). UTI was predominantly seen in age group 26-30 years followed by 21-25 years this was because maximum number of patients included in this study were from these age groups. This finding is comparable to studies by Bandyopadhyay et al[30] and Sabharwal[31] which reported prevalence of UTI as 25.2% and 24%, respectively. Study by Kant, et al [4]in rural Haryana also reported that the proportion of pregnant women with UTI was maximum in the third trimester similar to our study. This has also been shown in other studies in the past. Hence, if only one

time screening is affordable by the patient , then it should preferably done in the third trimester of pregnancy.

Table 8 shows the association of BV with UTI during pregnancy. BV was present in 42 antenatal patients (21%) and UTI was present in 32 patients (16%). About 15 patients with BV(out of 42) had associated UTI. BV associated with UTI was seen in 35.7% of the patients .The association between BV and UTI was found to be statistically significant ($p<0.05$). Previous similar study done by Lata et al[52] in Lucknow showed that about 14 patients with BV(out of 41) had associated UTI.. Also studies by Sharamiet al. and Hillerbrandet al[38]. have also reported pregnant women with bacterial vaginosis have a significantly increased risk of UTI.

The association between BV and UTI probably begins with raised vaginal pH because of the reduction in the number of vaginal lactobacilli. The lactobacilli produces lactic acid and hydrogen peroxide which are lethal to a number of bacterial species and also have been demonstrated to inhibit potential pathogens. In addition, lactobacilli produces a number of bacteriocins that are active against a wide range of bacteria and fungi. So, once the vaginal ecology is disturbed during BV, allowing colonization of potential uropathogens, the patient becomes more susceptible to UTI.

Sexual intercourse has an important confounding role on the association of BV and UTI[18]. An exogenous factor in the semen creates an imbalance in vaginal microflora and facilitate the development of BV. The urethral massage during

the sexual activity has a facilitating role in moving the urethral colonizers into the bladder[125]. Besides the effect of BV and sexual activity on the acquisition of UTI, the differences in the living standards, personal hygiene ,education, socio-cultural practices and awareness of the individual may be the reasons for the differences seen in the incidence of UTI in vaginitis in different communities.

Thus considering the complications associated with untreated BV and UTI it might be cost effective and wise to test for infectious vaginitis in women with UTI and vice versa.

Table 9 shows that about 4 patients with VVC(out of 23) had associated UTI. VVC associated with UTI was seen in 17.4% of the patients. However the association between VVC and UTI was statistically not significant ($p>0.05$). In contrast to our results study done by R Amatya et al [125]showed significant association between vaginal candidiasis with UTI during pregnancy.

In accordance with this study, vaginal colonization by E. coli, Klebsiella species, Proteus species., Staphylococci and Streptococci species during pregnancy has also been reported by different authors. Increase in vaginal pH with raised vascularity and estrogen content, bowel movement, cleansing habits, presence of hemorrhoids and use of sanitary napkins influence the growth and colonization of different pathogens in vagina during pregnancy[18]. In our study 17.5% of vaginal isolates were found as MDR which includes 28.5% of S.aureus and 23% of E.coli. This which was much lesser when compared to study done

by Lamichhane et al[18] in Nepal where 50% of vaginal isolates were multi drug resistant organisms. Streptococcus agalactiae(Group B Streptococci β haemolytic, GBS) constitutes about 2.5% of total vaginal isolates in our study.The isolate showed 100% susceptibility to cefotaxime, erythromycin, chloramphenicol and vancomycin. However study by Lamichhane et al[18] and M.Bayó et al[129] reported higher prevalence of 8.6% and 7% respectively. GBS infection is an important cause of neonatal morbidity and mortality. A complete and detailed evaluation of the vaginal microbiota with particular attention to the presence of potential pathogens such as GBS is a preventive strategy that can provide useful information to obstetricians and gynaecologist in managing the last days of pregnancy and delivery.

Table 13 shows the uropathogens isolated in our study which includes E.coli , Klebsiella spp, Proteus spp, A.baumannii, S.aureus and E.faecalis. About 33% of the urinary isolates were found MDR with 50% E.coli and K.pneumoniae being MDR. An isolate of A.baumannii from urine was also MDR. Lamichhaneet al[18] reported similar profile of uropathogens with 56% MDR isolates. The higher rate of MDR among uropathogens may be because of repeated and irrational use of antibiotics which is especially true for developing countries where antibiotics are irrationally prescribed not only by the medical practitioners but the antibiotics are also purchased directly from the pharmacist without any prescription. Therefore the empirical treatment of UTIs have become

more difficult and choice of antibiotics should be based on urine culture and antimicrobial susceptibility testing .

Table 18 and 19 shows that Candida albicans was the commonest species among candida isolated from HVS(60.9%) and Urine (80%) . Similar results were obtained by Holst et al[19] in a study conducted in Sweden and other studies conducted in our country.

C.tropicalis was the only Candida non albicans species isolated from (20%) urine specimen and it was also found in (13%) HVS .C.glabrata (13%), C.parapsilosis (8.6%) and C.krusei (4.3%) were the candida species isolated from HVS. Study by Gandhi el al[127] in Gujarat showed similar finding of C.albicans 66 %,C.glabrata 15%, C.krusei 3%,C.parapsilosis 5% and C.tropicalis 9.8% isolated from HVS.

Antifungal susceptibility pattern of candida isolates from HVS showed that Candida albicans isolates were 100% sensitive to Fluconazole ,Itraconazole and least sensitive to Nystatin B(86%). Among Candida non albicans species C.tropicalis and C.parapsilosis isolates were 100% sensitive to all azoles tested and Nystatin B. C.glabrata was only 33% sensitive to fluconazole ,itraconazole and C. krusei was 100% sensitive only to nystatin B as it is inherently resistant to azoles.These findings were in accordance with the study conducted by MondolS et al[128] which showed 82% of total Candida isolates were sensitive to fluconazole

by disc diffusion with highest rate of resistance among C.krusei (60%) followed by C.glabrata(30%) .

The resistance to fluconazole is of great concern because it is the azole which is most commonly used for superficial candidal infections as well as deep candidiasis . Since majority of the isolates from HVS and urine were C.albicans which were almost 100% susceptible to fluconazole its use may be continued for empirical therapy of uncomplicated candidal vulvo-vaginitis and UTI. However timely monitoring of drug susceptibility must be done to detect the emerging resistance.

Summary

SUMMARY

- Around 200 antenatal patients both OPD and IPD attendees of Institute of obstetrics and gynaecology who fulfilled the inclusion criteria were included in the study.
- The prevalence of infectious vaginitis among antenatal patients according to present study was 32.5% and it was most common in the age group 26-30years.
- Amsel's criteria and Nugent's score identified 17.5% and 21% of patients with BV respectively.
- BV was the most prevalent type of vaginitis (21%) followed by VVC(11.5%) and mixed infection was seen only in 3.5% of the patients.
- UTI was found to be 16% with higher rate of infection found in 3rd trimester (50%) predominantly seen in age group 26-30 years and the difference was found to be statistically significant($p<0.05$).
- BV associated with UTI was seen in 35.7% of the patients. The association between BV and UTI was found to be statistically significant($p<0.05$).
- VVC associated with UTI was seen in 17.4% of the patients. However the association between VVC and UTI was not statistically significant ($p>0.05$).
- About 17.5% of vaginal isolates were found to be MDR with 28.5% of S.aureus and 23% of E.coli being MDR organisms.

- About 33% of the urinary isolates were found as MDR which includes 50% E.coli and K.pneumoniae . An isolate of A.baumannii from urine was also MDR .
- Candida albicans was the commonest species among Candida isolated from HVS(60.9%) and Urine (80%) .
- C.tropicalis was the only Candida non albicans species isolated from(20%) urine specimen and it was also found in (13%) HVS. C.tropicalis both from HVS and urine was 100% sensitive to all azoles tested and Nystatin B.
- An isolate of Candida krusei inherently resistant to azoles but sensitive to Nystatin was isolated from HVS culture.

Conclusion

CONCLUSION

The conclusion from our study is that there is high prevalence of vaginitis among antenatal patients . Bacterial vaginosis was the most prevalent type of vaginitis in pregnant women which can contribute to adverse outcomes in pregnancy such as abortion ,premature rupture of membrane and preterm labor. Screening for BV among pregnant women at regular intervals or when symptomatic and an early treatment can not only reduce the adverse outcomes but also decrease perinatal and maternal morbidity and mortality. Therefore it is recommended that antenatal health care facilities should incorporate screening of vaginitis among pregnant women and those with BV should be screened for UTI. Since douching ,smoking and multiple sexual partners are risk factors of BV , preventive measures against these factors should also be undertaken.

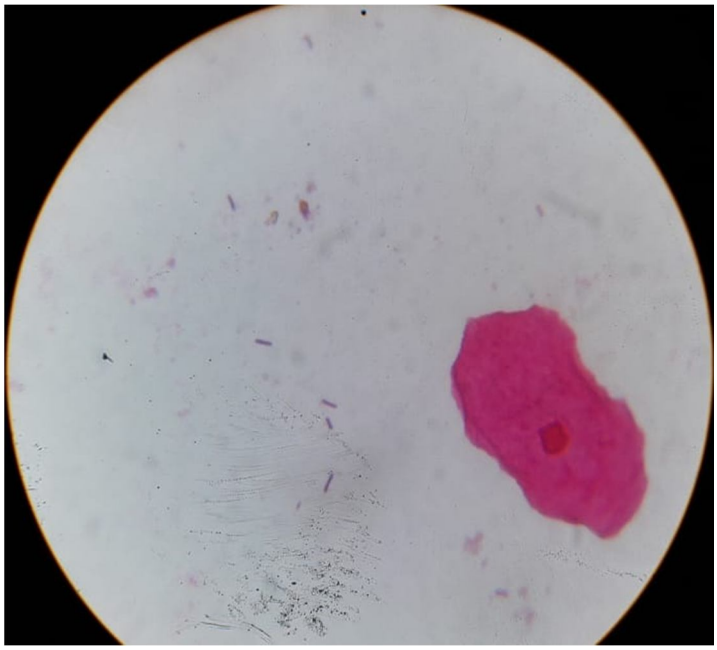
Increasing prevalence of MDR pathogens among urinary isolates (33%) makes the empirical treatment of UTIs more difficult and choice of antibiotics should be based on urine culture and antimicrobial susceptibility testing . Thus proper and judicious use of antibiotics must alsobe encouraged.

Further large scale studies are required to establish the association of UTI with infectious vaginitis in both pregnant and non pregnant women in order to identify the agents causing them so that prompt treatment can be initiated to prevent pregnancy associated complications in women of reproductive age group.

Colour Plates

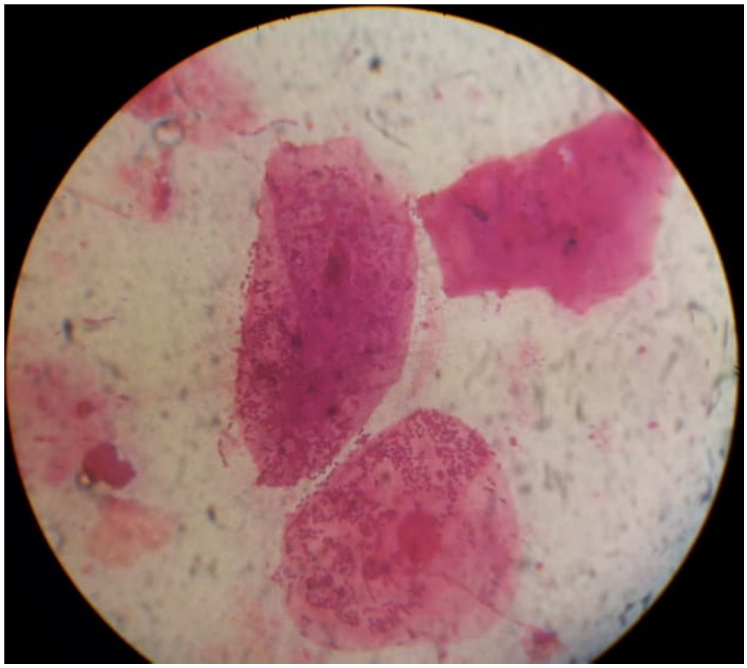
COLOUR PLATE 1

Direct Gram stain of HVS showing normal vaginal epithelial cell.



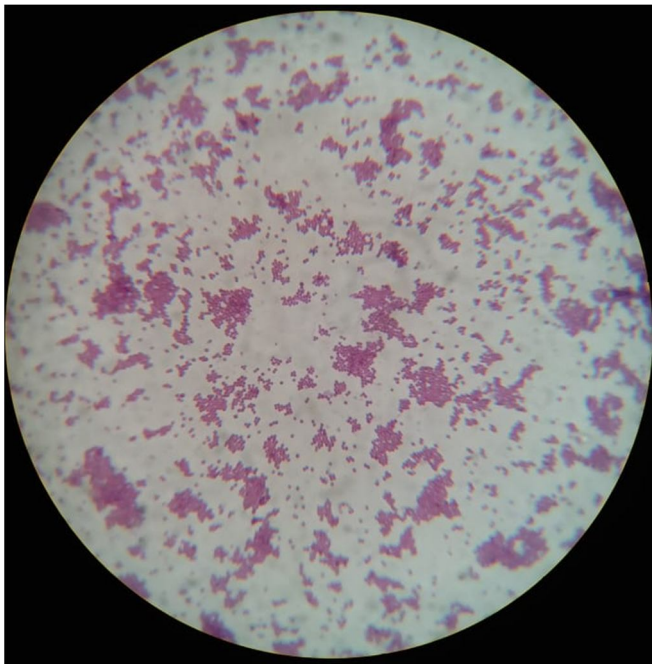
COLOUR PLATE 2

Direct Gram stain from HVS showing clue cells in a patient with Bacterial vaginosis



COLOUR PLATE 3

Culture smear showing GPC in clusters from a patient with Bacterial vaginosis

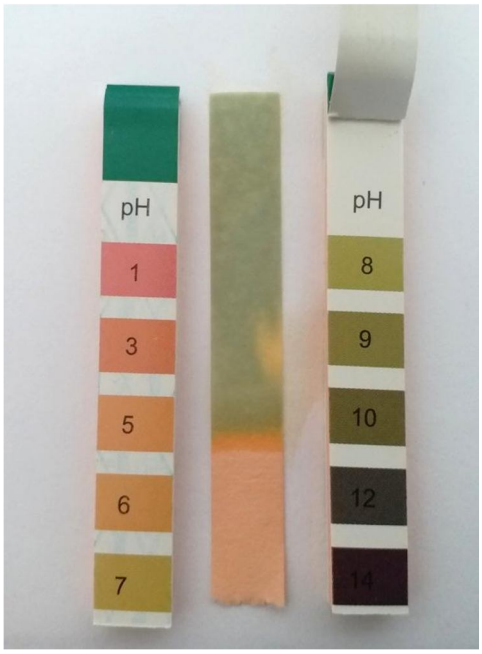


COLOUR PLATE 4

Direct Gram stain picture of a patient with vulvovaginal candidiasis

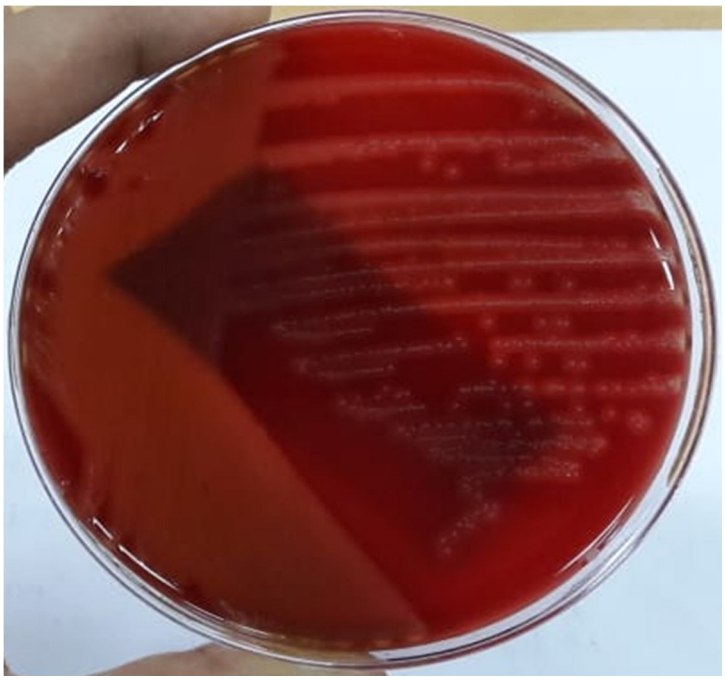
COLOUR PLATE 5

Vaginal pH measurement for Amsel's criteria : colour change corresponds to a pH between 8-9.



COLOUR PLATE 6

BAP showing βhemolytic colonies of S.agalactiae isolated from HVS sample.

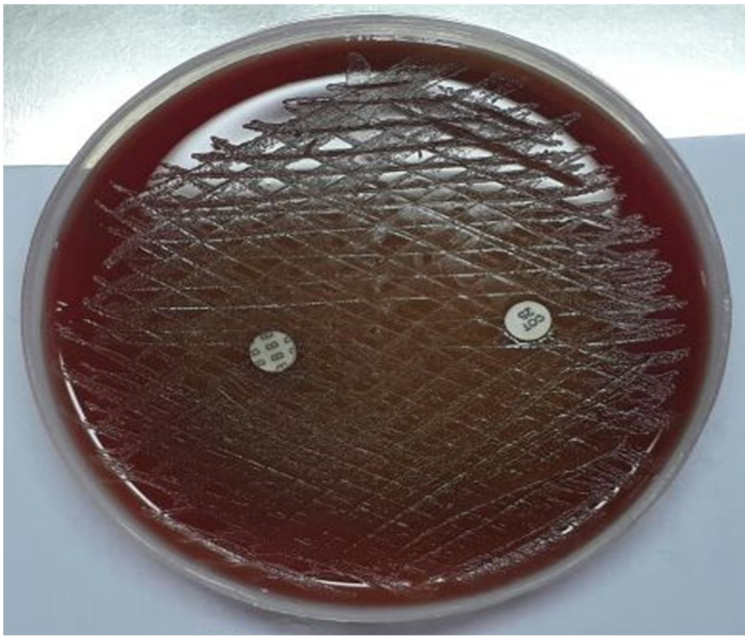


COLOUR PLATE 7

S.agalactiae showing positive CAMP test

COLOUR PLATE 8

S.agalactiae showing resistance to bacitracin and cotrimoxazole disc



COLOUR PLATE 9

CONS speciation by using novobiocin and polymyxin B disc

COLOUR PLATE 10

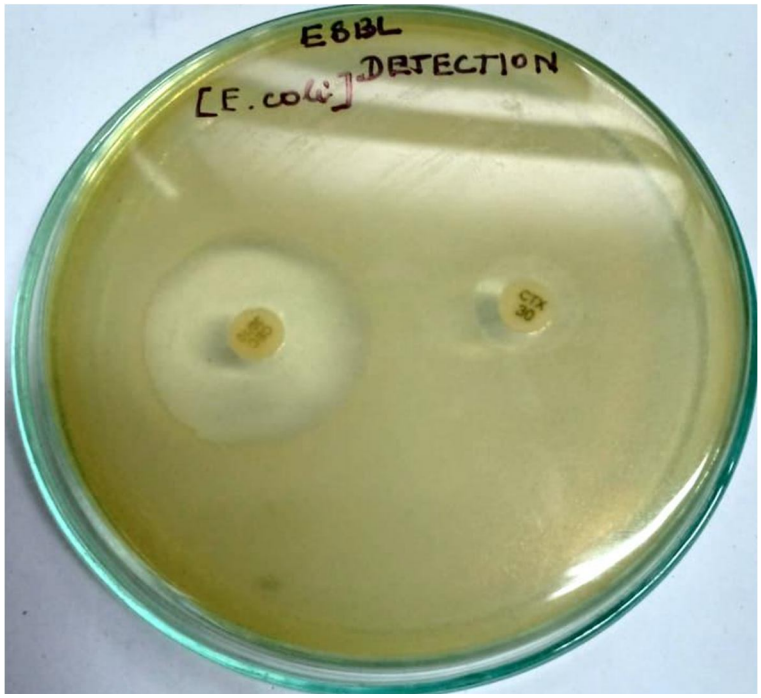
Biochemical reactions for E.coli

Tubes from left to right shows Indole - positive, TSI –Acid slant/acid butt, Citrate-negative,Urease- negative and Sugars: Glucose,lactose,sucrose,maltose and mannitol- fermented with acid and gas.

Lactose fermenting flat colonies of E.coli on CLED

COLOUR PLATE 11

ESBL production in E.coli

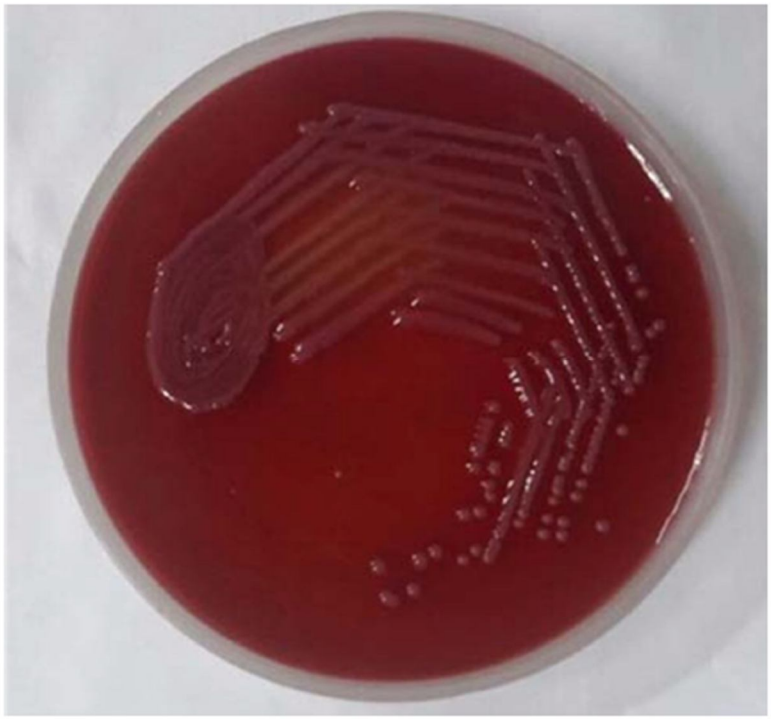


COLOUR PLATE 12

Biochemical reactions for K.pneumoniae

Tubes from left to right shows Indole - negative, TSI –Acid slant/acid butt with gas, Citrate-positive,Urease- positive and Sugars: Glucose,lactose,sucrose,maltose and mannitol- fermented with acid and gas.

Lactose fermenting mucoid colonies of K.pneumoniae on MAC



COLOUR PLATE 13

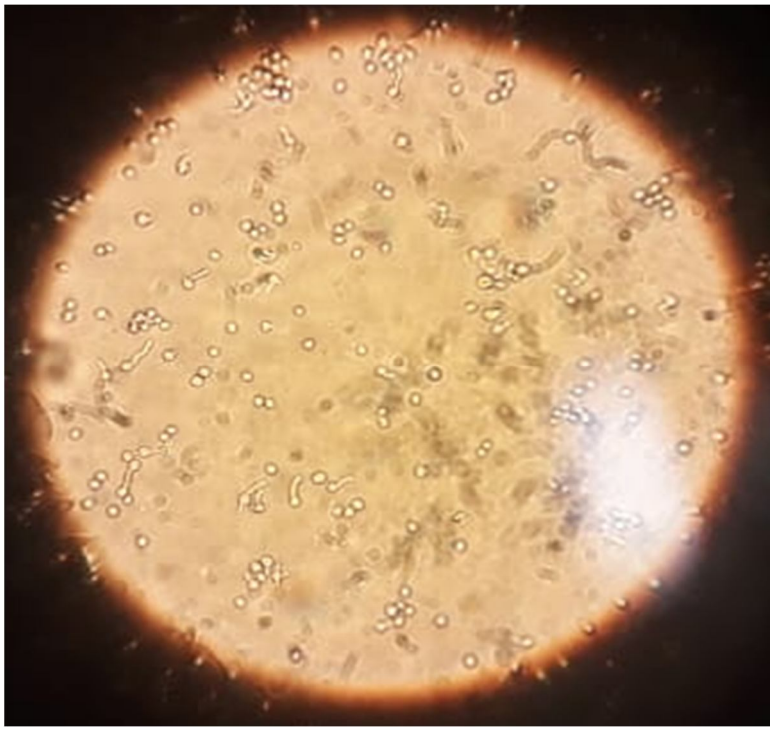
Vancomycin MIC by E test for MRSA strain

COLOUR PATE 14

SDA culture of Candida albicans showing cream coloured,smooth and pasty colonies

COLOUR PLATE 15

Candida albicans showing positive Germ tube test

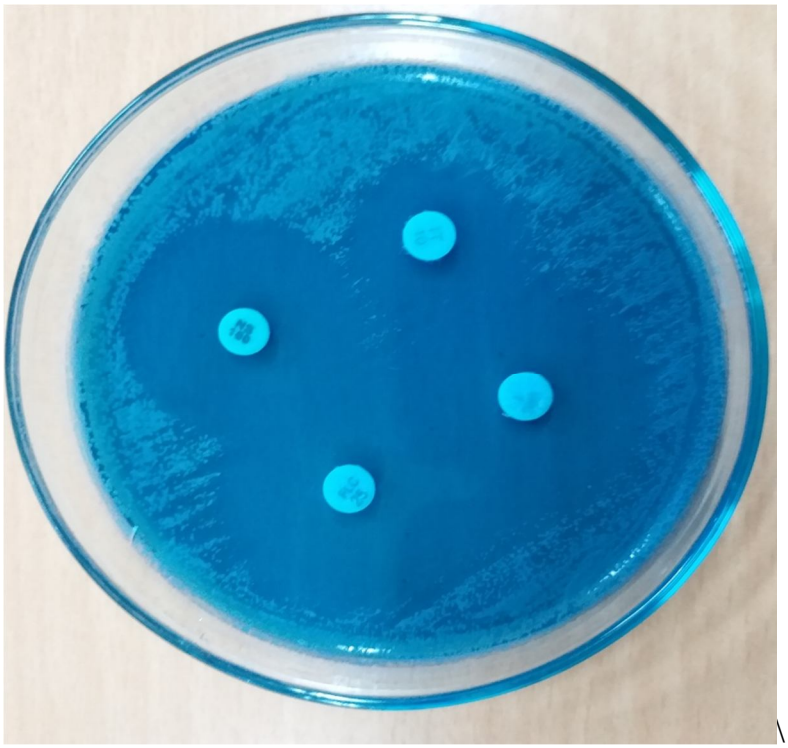


COLOUR PLATE 16

Sugar fermentation test showing reactions for C.albicans

COLOUR PLATE 17

Antifungal susceptibility by disc diffusion for Candida albicans



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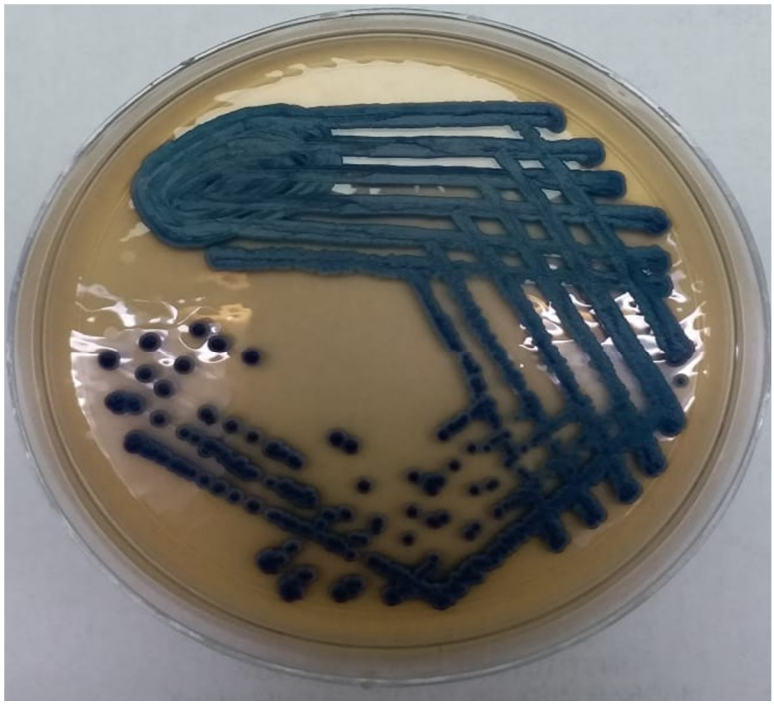
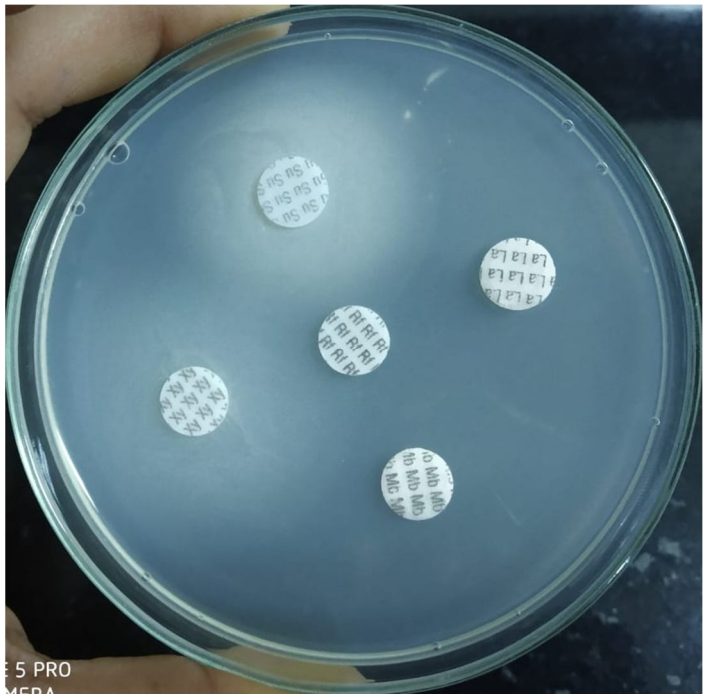
COLOUR PLATE 18

CHROM agar showing colonies of C.tropicalis



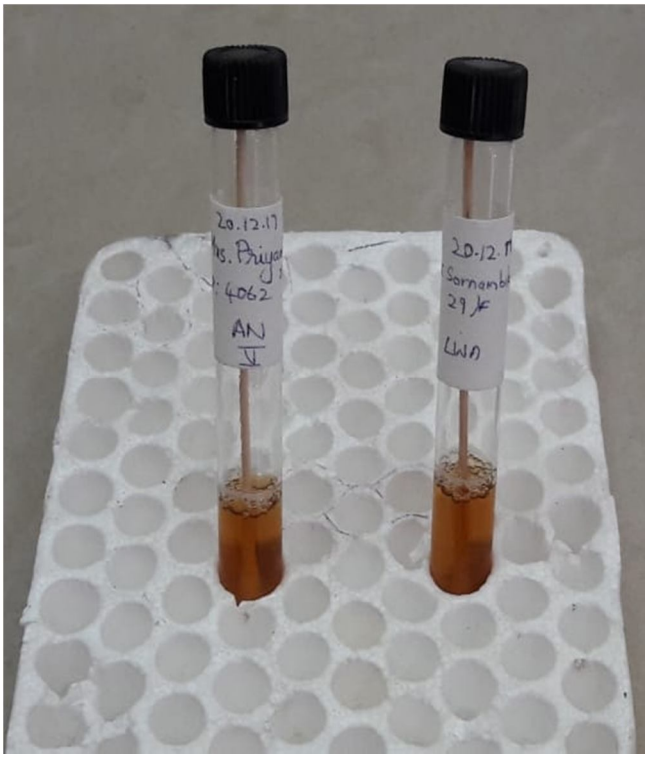
COLOUR PLATE 19

Sugar assimilation test showing reactions for C.tropicalis



COLOUR PLATE 20:

Modified Diamonds medium for culture of T.vaginalis



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Annexures

APPENDIX-1

ABBREVIATIONS

ATCC	-	American Type Culture Collection
BAP	-	Blood Agar Plate
BHI	-	Brain Heart Infusion
BV	-	Bacterial vaginosis
CA	-	Clavulanic acid
CAMP	-	Christie Atkins Munch Petersen
CDC	-	Center for Disease Control and Prevention
CFU	-	Colony forming unit
CLSI	-	Clinical laboratory standard Institute.
CLED	-	Cystine lactose electrolyte deficient agar
CONS	-	Coagulase Negative Staphylococcus aureus
DNA	-	Deoxyribonucleic acid
E test	-	Epsilon test (Epsilometer)
ELISA	-	Enzyme Linked Immuno Sorbant Assay
ESBL	-	Extended Spectrum Beta Lactamase
GNB	-	Gram Negative Bacilli
GPC	-	Gram Positive Bacilli
GMB	-	Glucose Methylene Blue medium
GWC	-	Greyish White Colonies
HVS	-	High vaginal swab
H_2S	-	Hydrogen sulphide

IUDs	-	Intra Uterine Devices
KOH	-	Potassium Hydroxide
MAC	-	Mac Conkey agar
MDR	-	Multi Drug Resistant
MHA	-	Mueller Hinton Agar
MIC	-	Minimum inhibitory concentration
MRSA	-	Methicillin resistant Staphylococcus aureus
MSSA	-	Methicillin sensitive Staphylococcus aureus
TSI	-	Triple Sugar Iron Agar
QC	-	Quality control
SDA	-	Sabourauds Dextrose Agar
STI	-	Sexually Transmitted Infections
TV	-	Trichomonas vaginalis
VVC	-	Vulvovaginal candidiasis
UTI	-	Urinary tract infection
UPEC	-	UroPathogenic Escherichia coli

ANTIBIOTICS

CTX	-	Cefotaxime
CEC	-	Cefotaxime+clavulanic acid
CX	-	Cefoxitin
IMP	-	Imipenem
AK	-	Amikacin
GM	-	Gentamicin
COT	-	Trimethoprim – Sulfamethaxozole
TET	-	Tetracycline
NIT	-	Nitrofurantoin
NOR	-	Norfloxacin
CAZ	-	Ceftazidime
PT	-	Piperacillin tazobactum
CIP	-	Ciprofloxacin
ERY	-	Erythromycin
HLG	-	High Level Gentamicin
PEN	-	Penicillin
VAN	-	Vancomycin
CK	-	Chloramphenicol
LZ	-	Linezolid
CD	-	Clindamycin

APPENDIX-II

STAINS, REAGENTS AND MEDIA

Gram staining:

- Methyl violet(2%)-10g of Methyl violet in 100 ml Absolute alcohol in 1 litre of Distilled water.(primary stain)
- Grams Iodine-10g Iodine in 20 g KI (fixative)
- Acetone-Decolourizing agent.
- Carbolfuchsin(1%)-Secondary stain.

MEDIA USED

1.MacConkey agar medium
Ingredients gram/liter

- Peptone 20g
- Lactose 10g
- NaCl 5.g
- Na- Deoxycholate 1.0
- Neutral Red 0.03
- Agar 15.0

Fifty-two grams of dehydrated MacConkey agar medium was suspended in 1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes.

2.Blood agar medium(5% sheep blood agar)
Ingredients gram/liter

- Peptone 10.00
- Distilled water 1 ltr.
- Sodium chloride 5.00
- Agar 15.00

Fourty grams of the dehydrated blood agar medium was suspended in 1000 mlcold distilled water in a flask and boiled to dissolve the medium completely. It was then sterilized by autoclaving at 121ºC and 15 lbs pressure for 15 minutes. The autoclaved materials were allowed to cool to a temperature of 45ºC in a water bath. Defibrinated5-10% sheep blood was then added to the medium aseptically and distributed to sterile petridishes. Sterile media was stored in refrigerator at 4ºC for future use.

3.Cystine lactose electrolyte deficient (CLED medium)
Ingredients gram/liter

- Peptone 4g
- Tryptone 4g
- Lab-Lemco powder 3g
- Lactose 10g

- *L*-cystine 0.128g
- bromothymol blue 0.02g
- agar 15g
- water 1L

Suspend the ingredients in the water, bring to the boil to dissolve. Sterilize by autoclaving at 121 ºC for 15minutes and mix well before pouring..
Store the plates at 2–8 ºC, preferably sealed in plastic bags to prevent loss of moisture. Shelf-life: Up to 4 weeks or longer provided there is no change in the appearance of the medium to suggest contamination or a change in pH.

4.Muller Hinton agar medium
Ingredients gram/liter

- Beef dehytrated infusion 300
- Casein hydrolysate 17.50
- Starch agar 1.50
- Agar 10.00

Thirty-eight grams of dehydrated Mueller Hinton agar medium was suspended in1000 ml cold distilled water and boiled to dissolve the medium completely. The solution was then sterilized by autoclaving at 121°C and 15 lbs pressure for 15 minutes. The autoclaved media was stored at 4°C.pH=7.4.

5.Sabouraud dextrose agar with antibiotics:

INGREDIENT	**Gm/ltr**
Peptone	10 gms
Dextrose	40 gms
Agar	20 gms
Distilled water	1000 gms
Gentamicin	20 mg

Final pH was adjusted to 5.6

The above ingredients were reconstituted in one litre of distilled water. Dissolve the powder water by boiling. Gentamicin is added to the boiling medium. The medium was then removed from heating ,mixed well and then dispersed in tubes and autoclaved at 121deg celcius for 15 minutes and the final pH was adjusted to 5.6.The tubes were cooled in slanted position and later the slants were stored in refrigerator.

6.Yeast nitrogen base medium (dehyrated media):

INGREDIENTS	GMS/L	INGREDIENTS	GMS/L
Ammonium sulphate	5.000	Niacin	0.0004
L-Histidine hydrochloride	0.010	p-Amino benzoic acid (PABA)	0.0002
DL-Methionine	0.020	Pyridoxine hydrochloride	0.0004
DL-Tryptophan	0.020	Riboflavin (Vitamin B2)	0.0002
Biotin	0.000002	Thiamine hydrochloride	0.0004
Calcium pantothenate	0.0004	Boric acid	0.0005
Folic acid	0.00002	Copper sulphate	0.00004
Inositol	0.002	Potassium iodide	0.0001
Ferric chloride	0.0002	Manganese sulphate	0.0004
Sodium molybdate	0.0002	Zinc sulphate	0.0004
Monopotassium phosphate	1.000	Magnesium sulphate	0.500
Sodium chloride	0.10	Calcium chloride	0.100

Dissolve 6.7 gms of media in 100 ml 0f distilled water. Serilise by filteration and store at 4 deg celcius.

7.CHROM agar candida medium:

INGREDIENTS	GMS/L
Peptone,	15 gms
Yeast extract	4 gms
Dipotassium hydrogen phosphate	1. gms
Chromogenic mixture	7.22 gms
Chloramphenicol	0.5 gms
Agar	15 gms

pH 6.3±0.2 at 25°C)

Suspend 42.72 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Do not autoclave. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION:

1.Catalase test:3% hydrogen peroxide

2.Oxidase reagent

Composition
Distilled water 10ml
Tetramethyl-P- phenylenedimine 0.1 g

3.Indole test
Composition
Ingredients amount

- Peptone 20g
- Sodium chloride 5g
- Distilled water 1 L
- After adjustment of the pH to 7.4 , sterilize by autoclaving at 1210C for 15 min.
- Kovac.s reagent
- Amyl or isoamyle alcohol 150ml
- *p* . Dimethyl-aminobenzaldehyde 10g
- Hydrochloric acid 50ml

Dissolve the aldehyde in the alcohol and slowly add the acid and store in the refrigerator.

4.Simmon's Citrate Medium:

- Koser's medium 1 ltr
- Agar 20g
- Bromothymol blue 0.2% 40ml
- Dispense, Autoclave at 121°for 15 min and allow to set as slopes.

5.Triple Sugar Iron medium:

- Beef extract 3g
- Yeast extract 3g
- eptone 20g
- Glucose 1g
- Lactose 10g
- Sucrose 10g
- Ferric citrate 0.3g
- Sodium chloride 5g
- Sodium thiosulphate 0.3g
- Agar 12g
- Phenol red 0.2% solution 12 ml
- Distilled water 1 ltr

Heat to dissolve the solids,add the indicator solution,mix and tube .Sterilize at 121° for15 min and cool to form slopes with deep butts.

6.Methyl Red test/Voges –Proskauer test:
A.MR/VP broth(Glucose broth/phosphate buffer broth)

- Polypeptone 7g
- Glucose 5g
- Dipotassium phosphate 5g
- Distilled water 1Ltr
- Final pH 6.9

B.Reagents

1. ∝-Napthol,5%(5gm in 100ml of absolute ethyl alcohol)
2. Potassium hydroxide 40%(Potassium hydroxide in 100ml of Distilled water).

7.Decarboxylase media:

Moller decarboxylase broth base:

- Ingredients gms/ml
- Peptone 5
- Beef extract 5
- Bromocresol purple 0.01
- Cresol red 0.005
- Glucose 0.5
- Pyridoxal 0.005
- Aminoacid

Add 10g of the levo form of the aminoacid for 1000 ml.mix and dispense in sterile tubes.

8.Hugh-Leifson's Oxidation-Fermentation test:

- Peptone 2g
- Sodium chloride 5g
- D-glucose 10g
- Bromothymol blue 0.03g
- Agar 3g
- Dipotassium phosphate 0.3g
- Distilled water 1ltr
- pH=7.1

Basal medium is autoclaved.1%of sterile sugar solutions is added to the basalmedium.Dispense into the sterile test tubes without slant.

ANNEXURE-I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.G.Jabeen Fathima
I Year PG in MD Microbiology
Institute of Microbiology
Madras Medical College
Chennai 600 003

Dear Dr.G.Jabeen Fathima,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY ON MICROBIOLOGICAL PROFILE OF VAGINITIS AND ITS ASSOCIATION WITH URINARY TRACT INFECTION DURING PREGNANCY IN A TERTIARY CARE HOSPITAL " - NO.17032017(I)**

The following members of Ethics Committee were present in the meeting hold on **02.03.2017** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr. K.Narayanasamy,MD,DM.,Dean(FAC), MMC,Ch-3	:Deputy Chairperson
3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4.Prof.S.Suresh, MS, Prof. of Surgery,MMC,Ch-3	: Member
5.Prof.Baby Vasumathi,MD.,Director, Inst. of O & G	: Member
6.Prof.K.Ramadevi,MD.,Director,Inst.of Bio-Che,MMC,Ch-3	: Member
7.Prof.R.Padmavathy, MD, Director,Inst.of Pathology,MMC,Ch-3	: Member
8.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
9.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
10.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE-II

PROFORMA

- Name : IP No:
- Age: Ward:
- Occupation: Contact No:
- Address:

- Presenting complaints

- Obstetric history: G P L A

- Personal history
- Past history
- Treatment history
- Any H/o symptoms in partner
- H/o multiple partners
- Clinical Diagnosis:

- Microbiological investigation:
- Direct Gram staining :
- Amsel criteria scoring :

- Nugent s criteria scoring:

- Wet mount examination:

- KOH examination:

- HVS culture: Mac conkeyagar:

  Blood agar:

  Sabouraud's dextrose agar:

  Diamond's media :

- Urine Culture : CLED Agar–

  Colony count –

- Organisms identified from HVS culture:

- Antibiotic sensitivity pattern –

- Organisms identified from urine culture:

- Antibiotic sensitivity pattern –

ANNEXURE-III

CONSENT FORM

STUDY TITLE :

A STUDY ON MICROBIOLOGICAL PROFILE OF VAGINITIS & ITS ASSOCIATION WITH URINARY TRACT INFECTION DURING PREGNANCY

IN A TERTIARY CARE HOSPITAL

I……, hereby give consent to participate in the study conducted by Dr.G.JabeenFathima, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my clinical Specimen (high vaginal swabs and urine sample) for further investigations.I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression Place Date

Of the patient/ relative

Patient Name & Address:

Signature of the investigator:

Signature of guide:

INFORMATION SHEET

STUDY TITLE :
A STUDY ON MICROBIOLOGICAL PROFILE OF VAGINITIS & ITS ASSOCIATION WITH URINARY TRACT INFECTION DURING PREGNANCY IN A TERTIARY CARE HOSPITAL

INVESTIGATOR : **Dr.G.JabeenFathima,**
Post Graduate,
Institute of Microbiology,
Madras Medical College,
Chennai - 600003

GUIDE : **Dr.Thasneem Banu S M.D.,**
Professor of Microbiology,
Institute of Microbiology,
Madras Medical College,
Chennai - 600003

- In pregnancy, normal vaginal microbiota, of lactobacilli is substituted by bacteria such as Gardnerellavaginalis, Mycoplasmahominis resulting in a significant reduction in lactobacilli & increased pH > 4 .5 which predispose to infection.
- Bacterial vaginosis (BV), vulvovaginal candidiasis (VC), and Trichomoniasis are responsible for 90% of cases of infectious vaginitis & it leads to obstetrical complications such as post abortion endometritis, chorioamnionitis, and premature labor .
- The most common infection in preterm delivery is BV.
- VC is an infection of the vagina caused by various species of Candida, a commensal fungus of the digestive and vaginal mucosae that become pathogenic under conditions such as pregnancy .
- Trichomoniasis is associated with PROM, premature delivery, LBW, postpartum endometritis, stillbirth, and neonatal death.
- UTI in women develop when uropathogens almost always from the fecal flora colonize the vagina, ascend into the bladder and in some cases the kidney

I am going tostudy on microbiological profile of vaginitis & its association with urinary tract infection during pregnancy in a tertiary care hospital .I am going to collect high vaginal swab and urine samples for this study and process them accordingly.200patients are included in this study after getting informed consent only. This study is entirely voluntary and patient can withdraw any time from this study. Extra cost will not be incurred to the patients in this study. Any doubt regarding this study will be willingly clarified. Results of the study will be published. In case of any doubt please contact Dr. G.JabeenFathima, Cell: 9894604826

சுய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு :

மூன்றாம் நிலை மருத்துவமனையில், கர்ப்பிணி பெண்களுக்கு யோனிப் பகுதியில் ஏற்படும் தொற்றுகளுக்கு காரணமான நுண்ணுயிர்களைப் பற்றியும் அதனுடன் தொடர்புடைய சிறுநீர் பாதை தொற்றையும் கண்டறியும் ஆய்வு.

பெயர் : வயது : தேதி :

பங்கேற்பாளர் எண் :

..... என்பவராகிய நான் இந்த ஆய்வின் விவரங்களும் அதன் நோக்கங்களும் முறையாக மருத்துவரிடம் கேட்டு அறிந்து கொண்டேன். எனது சந்தேகங்கள் அனைத்திற்கும் தகுந்த விளக்கம் அளிக்கப்பட்டது. இந்த ஆய்வில் முழு சுதந்திரத்துடன் மற்றும் சுயநினைவுடன் பங்கு கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன். இச்சுய ஒப்புதல் படிவத்தை பற்றி எனக்கு விளக்கப்பட்டது.

இந்த ஆய்வினை பற்றிய அனைத்து தகவல்களும் எனக்கு தெரிவிக்கப்பட்டது. இந்த ஆய்வில் எனது உரிமை மற்றும் பங்கினை பற்றி அறிந்து கொண்டேன்.

இந்த ஆய்வில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் நான் பங்கு பெறுகிறேன். இந்த ஆராய்ச்சியில் இருந்து நான் எந்நேரமும் பின் வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

இந்த ஆய்வில் கலந்து கொள்வதன் மூலம் என்னிடம் பெறப்படும் தகவலை ஆய்வாளர் இன்ஸ்டிட்யூசனல் எத்திக்ஸ் கமிட்டியினரிடமோ, அரசு நிறுவனத்திடமோ தேவைப்பட்டால் பகிர்ந்து கொள்ளலாம் என சம்மதிக்கிறேன்.

இந்த ஆய்வில் முடிவுகளை வெளியிடும்போது எனது பெயரோ, அடையாளமோ வெளியிடப்படாது என அறிந்து கொண்டேன். இந்த ஆய்வின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்று கொண்டேன். இந்த ஆய்விற்காக எனது சிறுநீர், உயர் யோனிப் பகுதியில் தடவல் மாதிரிகளை பரிசோதனை செய்துக் கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் பங்கேற்கும் பொழுது ஏதேனும் சந்தேகம் ஏற்பட்டால், உடனே ஆய்வாளரை தொடர்பு கொள்ள வேண்டும் என அறிந்து கொண்டேன்.

இச்சுய ஒப்புதல் படிவத்தில் கையெழுத்திடுவதன் மூலம் இதிலுள்ள அனைத்து விஷயங்களும் எனக்கு தெளிவாக விளக்கப்பட்டது என்றும் தெரிவிக்கிறேன். இச்சுய ஒப்புதல் படிவத்தின் ஒரு நகல் எனக்கு கொடுக்கப்படும் என்றும் தெரிந்து கொண்டேன்.

பங்கேற்பாளர் / (பங்கேற்பாளரின் பெற்றோர்)
கையொப்பம் / இடது பெருவிரல் ரேகை தேதி :

ஆய்வாளர் கையொப்பம் தேதி :

MASTER CHART

Sample no.	IP/OP	Age	Trimester	Discharge	Foul smell	Lower Abdominal pain	Burning micturation	vaginal discomfort	wet mount	INFECTIOUS VAGINITIS					AK	GM	CIP	CTX	CAZ	COT	CEC	PEN	ERY	CX	TET	PT	CD	IMP	LZ	VAN	CK	URINARY ISOLATE	GM	NIT	NOR	CTX	CAZ	AK	CIP	PT	PEN	ERY	CX	COT	HLG	CEC	IMP	TET
										BACTERIAL		Trichomonas vaginalis	Vulvo - Vaginal Candidiasis	Bacterial Isolate																																							
										Amsel's criteria	Nugent's criteria																																											
1	IP	35	3	+	+	-	-	-	-	NO BV	NO BV	-	candida albicans	NG																		NG																		
2	IP	20	1	+	-	+	-	-	-	NO BV	NO BV	-	-	K.pneumoniae	S	S	S	S		S	S											NG																		
3	OP	26	2	+	-	+	-	+	-	NO BV	NO BV	-	-	K.oxytoca (ESBL)	S	S	S	R		R	S											NG																		
4	IP	23	3	-	-	+	+	-	-	NO BV	NO BV	-	-	NG																		$E.coli\ 10^5$ (ESBL)	S	S	S	R		S									S				
5	IP	23	1	+	-	-	-	-	-	NO BV	BV	-	-	NG																		NG																		
6	IP	26	3	+	+	+	+	-	Clue cells	BV	BV	-	-	P.mirabllis	S	S	R		S	S						S					$P.mirablis1\ 0^5$	S		S		S	S	S	S									
7	IP	28	3	-	-	+	-	+	-	NO BV	NO BV	-	-	NG																		NG																		
8	OP	25	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
9	OP	29	3	+	-	-	+	-	-	BV	BV	-	-	NG																		$E.coli\ 10^5$	S	S	S	S		S									S				
10	IP	22	2	+	-	-	+	+	-	NO BV	NO BV	-	candida albicans	NG																		$Candida\ albicans10^5$																		
11	IP	25	1	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
12	IP	20	3	-	-	+	-	+	-	NO BV	NO BV	-	-	NG																		NG																		
13	IP	30	3	+	+	+	+	-	Clue cells	BV	BV	-	-	K.pneumoniae (ESBL)	S	S	S	R		S	S											$P.mirablis1\ 0^5$	S		S		R	S	S	S									
14	IP	28	3	+	-	-	+	-	Clue cells	BV	BV	-	candida non albicans	NG																		$K.oxytoca1\ 0^5$(ESBL)	S	S	S	R		S									S				
15	IP	19	2	+	-	+	-	+	-	NO BV	NO BV	-	-	S.aureus (MSSA)			S			S		S	S	S			S					NG																		
16	OP	21	2	+	+	-	-	-	Clue cells	BV	BV	-	-	NG																		NG																		
17	OP	25	2	+	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
18	IP	26	3	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
19	IP	33	3	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
20	IP	40	3	+	+	-	-	-	Clue cells	BV	BV	-	candida non albicans	NG																		NG																		
21	IP	22	1	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
22	IP	30	2	-	-	-	+	-	-	NO BV	BV	-	-	NG																		$Acinetobacter\ spp10^5$	R				R		R	S				R				R
23	IP	29	2	+	-	+	-	+	Clue cells	BV	BV	-	-	E.coli(ESBL)	S	S	S	R		R	S											NG																		
24	IP	20	3	+	-	+	-	-	-	BV	BV	-	-	E.coli	S	S	S	S		S	S											NG																		
25	IP	29	2	+	+	-	-	-	Clue cells	BV	BV	-	candida albicans	NG																		NG																		
26	IP	20	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
27	IP	20	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
28	IP	24	2	+	+	+	-	-	Clue cells	BV	BV	-	-	S.aureus (MSSA)			S			R		S	S	S			S					NG																		
29	OP	25	3	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
30	OP	21	3	+	-	-	+	-	-	NO BV	BV	-	-	NG																		$S.aureus10^5$ (MSSA)		S					S		S	S	S	S				
31	IP	31	2	+	+	-	-	-	Clue cells	BV	NO BV	-	-	NG																		NG																		
32	IP	29	1	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		

Sample no.	IP/OP	Age	Trimester	Discharge	Foul smell	Lower Abdominal pain	Burning micturation	vaginal discomfort	wet mount	INFECTIOUS VAGINITIS					AK	GM	CIP	CTX	CAZ	COT	CEC	PEN	ERY	CX	TET	PT	CD	IMP	LZ	VAN	CK	URINARY ISOLATE	GM	NIT	NOR	CTX	CAZ	AK	CIP	PT	PEN	ERY	CX	COT	HLG	CEC	IMP	TET
										BACTERIAL		Trichomonas vaginalis	Vulvo - Vaginal Candidiasis	Bacterial Isolate																																							
										Amsel's criteria	Nugent's criteria																																										
33	OP	36	1	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
34	IP	28	2	-	-	-	+	-	-	NO BV	NO BV	-	-	NG																		NG																	
35	IP	27	2	+	+	-	-	-	Clue cells	BV	NO BV	-	-	NG																		NG																	
36	IP	26	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
37	IP	24	3	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
38	IP	25	2	-	-	+	-	+	-	NO BV	NO BV	-	-	S.aureus (MSSA)			S			S		R	S	S			S					NG																	
39	IP	30	3	+	-	-	+	-	-	NO BV	NO BV	-	-	NG																		S.aureus10^5 (MSSA)		S				S		R	S	S	R					
40	IP	21	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
41	IP	23	2	+	-	-	-	+	-	NO BV	NO BV	-	-	NG																		NG																	
42	OP	24	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
43	OP	26	2	+	-	+	+	-	-	BV	NO BV	-	-	K.pneumoniae	S	S	S	S		S	S										K.pneumoniae10^5	S	S	S	S		S								S			
44	IP	27	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
45	IP	32	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
46	IP	28	2	+	-	+	-	+	-	NO BV	BV	-	-	P.mirablis	S	S	S		S	R						S					E.coli 10^4	S	S	R	S		S								S			
47	OP	25	3	+	+	-	-	-	Clue cells	BV	BV	-	candida albicans	NG																		NG																	
48	OP	25	1	+	-	-	+	-	-	NO BV	NO BV	-	-	NG																		NG																	
49	IP	28	1	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
50	IP	20	2	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
51	OP	29	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
52	IP	21	1	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
53	IP	25	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
54	IP	26	2	+	-	-	-	-	-	NO BV	BV	-	-	NG																		NG																	
55	IP	25	2	+	-	+	-	-	-	NO BV	NO BV	-	-	Strep.agalactiae				S				R	S				S			S	S	NG																	
56	IP	22	3	+	-	-	+	+	-	BV	BV	-	-	E.coli	S	S	S	S		S	S										E.coli10^5	S	S	S	S		S								S			
57	IP	30	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
58	IP	20	2	+	+	-	-	-	-	NO BV	NO BV	-	candida albicans	NG																		NG																	
59	OP	25	2	+	-	-	-	-	-	NO BV	NO BV	-	candida albicans	NG																		NG																	
60	IP	30	2	+	-	+	+	-	Clue cells	BV	BV	-	-	S.aureus (MRSA)			R			R		R	R	R	R		R		S		S	Candida non albicans10^5																	
61	IP	30	3	+	-	-	-	-	-	NO BV	NO BV	-	-	K.oxytoca	S	S	R	S		R	S										E.coli10^5	R	S	S	S		S								S			
62	OP	21	1	+	-	+	-	-	-	NO BV	NO BV	-	-	S.aureus (MSSA)			R			S		R	S	S			S					NG																	
63	OP	25	3	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
64	OP	31	2	+	-	-	+	-	-	NO BV	NO BV	-	-	K.oxytoca	S	S	S	S		S	S										K.pneumoniae10^3	R	S	S	S		S								S			
65	IP	20	3	-	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
66	IP	27	2	+	-	-	-	-	-	NO BV	BV	-	-	E.coli(ESBL)	S	S	S	R		S	S										NG																	
67	IP	22	3	-	-	+	-	+	-	NO BV	NO BV	-	-	E.coli(ESBL)	R	S	R	R		R	R				S				S			NG																	
68	IP	23	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	

Sample no.	IP/OP	Age	Trimester	Discharge	Foul smell	Lower Abdominal pain	Burning micturation	vaginal discomfort	wet mount	INFECTIOUS VAGINITIS					AK	GM	CIP	CTX	CAZ	COT	CEC	PEN	ERY	CX	TET	PT	CD	IMP	LZ	VAN	CK	URINARY ISOLATE	GM	NIT	NOR	CTX	CAZ	AK	CIP	PT	PEN	ERY	CX	COT	HLG	CEC	IMP	TET
										BACTERIAL		Trichomonas vaginalis	Vulvo - Vaginal Candidiasis	Bacterial Isolate																																						
										Amsel's criteria	Nugent's criteria																																										
69	IP	24	1	+	+	-	+	-	-	NO BV	NO BV	-	-	NG																		NG																	
70	IP	25	2	+	+	-	-	-	Clue cells	BV	NO BV	-	candida non albicans	NG																		NG																	
71	IP	24	3	-	-	-	+	-	-	NO BV	BV	-	-	NG																		E.coli10^3	R	S	R	S		S								S			
72	IP	20	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
73	IP	25	2	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
74	IP	25	3	+	-	-	-	-	-	BV	BV	-	-	NG																		NG																	
75	IP	24	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
76	IP	29	2	+	-	+	-	-	-	NO BV	NO BV	-	-	K.pneumoniae	S	S	R	S		S	S										NG																	
77	IP	21	3	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
78	IP	23	1	+	-	-	-	+	-	NO BV	BV	-	-	NG																		NG																	
79	IP	22	2	+	-	-	+	-	-	NO BV	NO BV	-	-	NG																		P.mirablis10^5	S		S		S	S	R	S									
80	IP	27	1	+	-	-	-	-	Clue cells	BV	BV	-	-	NG																		NG																	
81	IP	22	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
82	IP	21	2	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
83	IP	34	2	+	+	-	-	-	-	BV	BV	-	candida albicans	NG																		NG																	
84	OP	24	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
85	IP	26	2	+	+	-	-	-	Clue cells	BV	BV	-	-	NG																		NG																	
86	OP	34	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
87	IP	25	3	+	-	+	-	-	-	BV	BV	-	-	NG																		NG																	
88	IP	24	1	+	+	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
89	IP	34	2	+	-	-	+	-	-	NO BV	NO BV	-	-	NG																		NG																	
90	IP	22	1	+	-	-	-	-	-	BV	BV	-	candida non albicans	NG																		NG																	
91	IP	23	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
92	IP	29	3	+	-	-	-	-	-	NO BV	NO BV	-	candida albicans	NG																		NG																	
93	IP	23	1	+	+	-	-	-	Clue cells	BV	BV	-	-	NG																		P.aeruginosa10^5	S		S		S	S	S	S									
94	IP	23	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
95	IP	29	3	-	-	+	+	-	-	NO BV	NO BV	-	-	E.coli (ESBL)	S	S	R	R		R	S										NG																	
96	IP	28	3	+	-	-	+	-	-	NO BV	NO BV	-	-	NG																		E.coli10^5(ESBL)	S	S	S	R		S								S			
97	IP	26	2	-	-	+	-	-	Clue cells	NO BV	NO BV	-	-	K.oxytoca (ESBL)	S	S	R	R		R	S										NG																	
98	IP	32	3	+	-	-	-	+	-	NO BV	BV	-	-	S.aureus (MSSA)			S			S		S	S	S			S					NG																	
99	IP	25	2	+	-	-	-	-	-	NO BV	BV	-	-	NG																		K.oxytoca10^5(ESBL)	S	S	S	R		S								S			
100	IP	31	2	+	-	-	-	-	-	NO BV	NO BV	-	-	CONS(MS)			R			S		S	S	S			S					NG																	
101	IP	28	3	+	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
102	IP	23	2	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
103	IP	25	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	

Sample no.	IP/OP	Age	Trimester	Discharge	Foul smell	Lower Abdominal pain	Burning micturation	vaginal discomfort	wet mount	INFECTIOUS VAGINITIS					AK	GM	CIP	CTX	CAZ	COT	CEC	PEN	ERY	CX	TET	PT	CD	IMP	LZ	VAN	CK	URINARY ISOLATE	GM	NIT	NOR	CTX	CAZ	AK	CIP	PT	PEN	ERY	CX	COT	HLG	CEC	IMP	TET
										BACTERIAL		Trichomonas vaginalis	Vulvo - Vaginal Candidiasis	Bacterial Isolate																																						
										Amsel's criteria	Nugent's criteria																																										
104	IP	42	2	+	+	-	-	-	-	NO BV	NO BV	-	-	NG																		E.coli10^5(ESBL)	R	S	R	R		S								S				
105	IP	15	1	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
106	IP	24	3	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
107	IP	22	3	+	-	-	-	-	-	NO BV	NO BV	-	candida albicans	NG																		NG																		
108	IP	23	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
109	IP	24	1	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
110	IP	25	2	+	+	+	-	+	-	NO BV	NO BV	-	-	K.oxytoca	R	R	S	S		S	S										NG																		
111	IP	24	2	-	-	+	-	-	-	NO BV	NO BV	-	-	E.coli	S	S	S	S		S	S										NG																		
112	OP	23	3	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
113	IP	26	2	+	-	-	+	-	-	NO BV	BV	-	-	NG																		K.pneumoniae10^5	R	R	R	R		R								R	S	S
114	IP	30	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
115	IP	20	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
116	IP	32	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
117	IP	22	3	-	_	-	+	-	-	NO BV	NO BV	-	-	NG																		NG																		
118	IP	25	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
119	IP	23	2	+	+	-	+	-	Clue cells	BV	BV	-	-	NG																		K.oxytoca10^3	R	S	S	S		R								S		
120	OP	24	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
121	IP	25	2	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
122	IP	26	1	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
123	IP	24	3	+	-	+	-	-	Clue cells	BV	BV	-	-	K.pneumoniae	S	S	R	S		R	S										NG																		
124	IP	26	2	+	+	-	-	-	-	NO BV	NO BV	-	candida non albicans	NG																		NG																		
125	IP	33	1	+	-	-	-	-	-	BV	NO BV	-	-	NG																		NG																		
126	IP	34	1	-	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
127	IP	27	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		P.aeruginosa10^5	S		R		S	S	R	S								
128	IP	26	3	+	-	-	+	-	-	NO BV	NO BV	-	-	NG																		NG																		
129	IP	23	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
130	IP	27	2	+	+	+	-	-	Clue cells	BV	BV	-	-	E.coli	S	S	S	S		R	S										Candida albicans10^5																		
131	IP	33	1	+	-	-	-	+	-	NO BV	NO BV	-	-	NG																		NG																		
132	IP	25	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
133	OP	23	1	+	-	-	-	-	-	NO BV	NO BV	-	candida non albicans	NG																		NG																		
134	IP	28	2	+	-	-	-	+	-	NO BV	NO BV	-	candida non albicans	NG																		NG																		
135	IP	21	3	+	-	-	-	-	Clue cells	NO BV	NO BV	-	-	NG																		NG																		
136	IP	24	3	+	+	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		

Sample no.	IP/OP	Age	Trimester	Discharge	Foul smell	Lower Abdominal pain	Burning micturation	vaginal discomfort	wet mount	INFECTIOUS VAGINITIS					AK	GM	CIP	CTX	CAZ	COT	CEC	PEN	ERY	CX	TET	PT	CD	IMP	LZ	VAN	CK	URINARY ISOLATE	GM	NIT	NOR	CTX	CAZ	AK	CIP	PT	PEN	ERY	CX	COT	HLG	CEC	IMP	TET
										BACTERIAL		Trichomonas vaginalis	Vulvo - Vaginal Candidiasis	Bacterial Isolate																																						
										Amsel's criteria	Nugent's criteria																																										
137	IP	28	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
138	IP	19	2	-	-	+	+	-	-	NO BV	NO BV	-	-	NG																		NG																	
139	IP	24	2	-	-	+	+	-	-	NO BV	NO BV	-	-	NG																		NG																	
140	IP	28	3	+	-	-	-	-	-	NO BV	NO BV	-	candida albicans	NG																		NG																	
141	IP	23	3	+	+	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
142	IP	24	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
143	OP	28	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
144	IP	29	3	+	-	-	-	-	Clue cells	BV	BV	-	-	NG																		NG																	
145	OP	36	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
146	OP	22	3	+	-	-	-	-	-	BV	BV	-	-	NG																		Candida albicans10^5																	
147	OP	33	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
148	IP	18	1	+	-	-	-	+	Clue cells	BV	BV	-	candida albicans	NG																		Enterococcus spp10^4		S					S		R	S			S			S
149	OP	20	3	+	+	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
150	OP	29	2	-	-	+	+	-	-	NO BV	NO BV	-	-	NG																		NG																	
151	IP	36	2	-	-	+	+	-	-	NO BV	NO BV	-	-	NG																		NG																	
152	IP	25	2	+	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
153	IP	21	1	-	-	+	+	-	-	NO BV	NO BV	-	-	NG																		NG																	
154	IP	27	3	+	+	-	-	+	-	NO BV	NO BV	-	candida non albicans	NG																		K.pneumoniae10^5	S	R	S	R		R							S				
155	IP	23	3	-	-	+	-	+	-	NO BV	NO BV	-	-	NG																		NG																	
156	IP	24	2	+	-	-	-	-	-	NO BV	BV	-	-	K.pneumoniae	S	S	S	R			R				S			S				NG																	
157	IP	20	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		E.coli10^5	R	R	R	R		S							R	S	S
158	OP	26	1	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
159	OP	30	2	+	-	-	+	-	-	NO BV	NO BV	-	-	NG																		NG																	
160	IP	30	2	+	+	-	-	+	-	BV	BV	-	-	E.coli (ESBL)	S	S	R	R		S	S											E.coli10^5	R	S	R	R		S							S		
161	IP	31	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		p.mirablis10^3	S		S		R	S	S	S							
162	IP	31	2	+	-	-	-	-	-	NO BV	NO BV	-	-	K.oxytoca	S	S	R	S		S	S											NG																	
163	IP	22	3	+	-	-	-	-	-	NO BV	NO BV	-	-	E.coli (ESBL)	S	S	S	R		R	S											E.coli10^5	S	S	R	R		S							R		
164	IP	28	1	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
165	IP	25	2	-	-	+	+	-	-	NO BV	NO BV	-	-	NG																		NG																	
166	IP	18	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
167	IP	27	3	+	-	-	-	-	Clue cells	BV	BV	-	-	NG																		K.pneumoniae10^5	S	S	R	S		S							S		
168	OP	30	2	+	-	-	+	+	-	NO BV	NO BV	-	candida albicans	NG																		Candida albicans10^5																	
169	OP	15	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																	
170	OP	24	2	+	-	-	+	-	-	NO BV	BV	-	-	E.coli (ESBL)	S	S	S	R		S	S											E.coli10^3	S	S	R	S		S							S		
171	IP	22	2	-	-	+	-	+	-	NO BV	NO BV	-	candida non albicans	NG																		NG																	

Sample no.	IP/OP	Age	Trimester	Discharge	Foul smell	Lower Abdominal pain	Burning micturation	vaginal discomfort	wet mount	INFECTIOUS VAGINITIS					AK	GM	CIP	CTX	CAZ	COT	CEC	PEN	ERY	CX	TET	PT	CD	IMP	LZ	VAN	CK	URINARY ISOLATE	GM	NIT	NOR	CTX	CAZ	AK	CIP	PT	PEN	ERY	CX	COT	HLG	CEC	IMP	TET
										BACTERIAL		Trichomonas vaginalis	Vulvo - Vaginal Candidiasis	Bacterial Isolate																																								
										Amsel's criteria	Nugent's criteria																																												
172	IP	25	1	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
173	IP	26	3	+	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
174	IP	26	2	+	-	-	-	-	-	NO BV	NO BV	-	-	P.mirablis	S	S	S		S	S						S						NG																		
175	IP	27	2	+	-	-	-	-	-	NO BV	NO BV	-	candida albicans	NG																		NG																		
176	IP	30	2	+	-	-	-	-	-	NO BV	NO BV	-	candida albicans	NG																		NG																		
177	IP	36	1	-	-	+	+	+	-	NO BV	NO BV	-	-	NG																		NG																		
178	IP	37	3	+	-	+	-	-	-	NO BV	NO BV	-	-	NG																		E.coli10^{3}	S	R	S	R		S										R		
179	IP	33	3	+	-	+	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
180	IP	27	3	+	-	-	-	-	-	BV	NO BV	-	-	NG																		NG																		
181	IP	31	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
182	IP	23	2	+	-	-	-	-	-	NO BV	NO BV	-	-	K.pneumoniae (ESBL)	S	S	S	R		S	S											K.oxytoca10^{2}	S	R	S	S		S								S		
183	IP	21	1	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
184	IP	23	1	-	-	+	+	-	-	NO BV	NO BV	-	-	NG																		NG																		
185	IP	24	3	+	+	-	-	-	Clue cells	BV	NO BV	-	-	NG																		NG																		
186	IP	20	2	-	-	+	-	+	-	NO BV	NO BV	-	-	E.coli (ESBL)	S	S	R	R		R	S											NG																		
187	IP	23	2	+		-	+	-	-	NO BV	NO BV	-	-	K.oxytoca (ESBL)	S	S	S	R		S	S											K.oxytoca10^{5}	S	S	S	R		S								S		
188	IP	25	2	+	-	-	+	-	-	NO BV	NO BV	-	-	CONS(MS)			S			R		R	R	S			S					s.aureus10^{3} (MSSA)		S					S		S	S	S	S				
189	IP	19	2	+	-	-	-	-	-	NO BV	BV	-	-	NG																		NG																		
190	IP	20	2	-	-	+	+	-	-	NO BV	NO BV	-	-	NG																		NG																		
191	IP	31	3	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
192	IP	27	2	+	+	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
193	IP	20	3	+	+	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
194	IP	18	1	+	-	+	-	+	-	NO BV	NO BV	-	-	NG																		NG																		
195	IP	25	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
196	IP	24	2	+	-	+	+	-	-	NO BV	NO BV	-	-	S.aureus(MRSA)			S			R		R	R	R	R		R		S		S	P.aeruginosa10^{3}		S	S		R	S		S								
197	IP	20	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		S.aureus10^{5} (MSSA)		S					R		R	R	S	R				
198	IP	21	2	-	-	-	+	-	-	NO BV	NO BV	-	-	NG																		Enterococcus spp10^{5}		S					R		S	R			S			S
199	IP	35	2	+	-	-	-	-	-	NO BV	NO BV	-	-	NG																		NG																		
200	IP	21	2	-	-	+	+	-	-	NO BV	NO BV	-	-	E.coli(ESBL)	S	S	S	R		S	S											E.coli10^{3}	S	S	S	S		S								S		